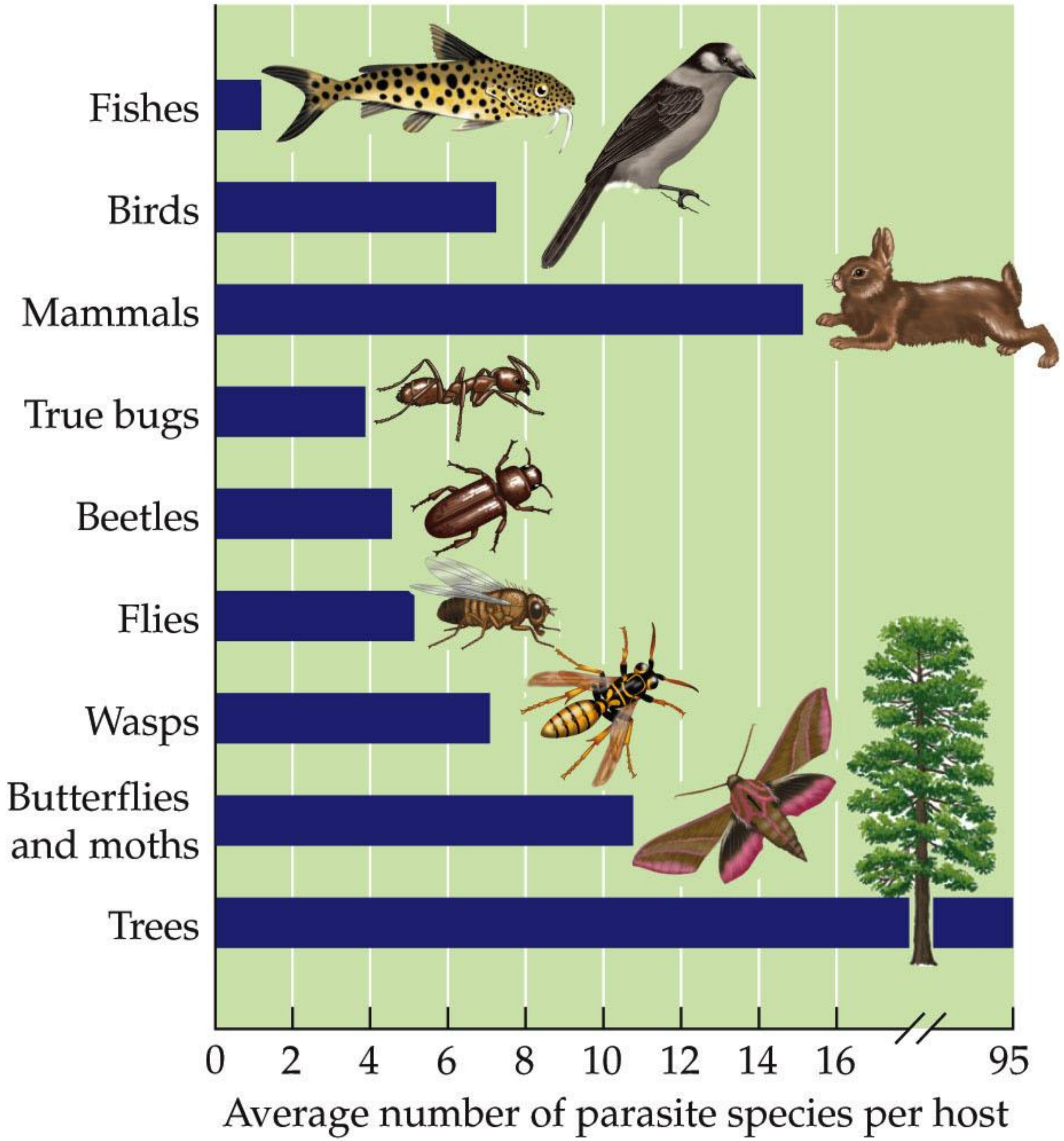
Коммуникация:

клетка-хозяин ← → внутриклеточный паразит (клетка)

с/к «Межклеточная коммуникация»

Лекция 10

Many Species Are Host to More Than One Parasite Species



ECOLOGY, Figure 13.4

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Coevolution

Host and parasite populations can evolve together, each in response to selection imposed by the other.

When a parasite and its host each possess specific adaptations, it suggests that the strong selection pressure hosts and parasites impose on each other has caused both of their populations to evolve.

Бактерии: Размеры бактериальных клеток

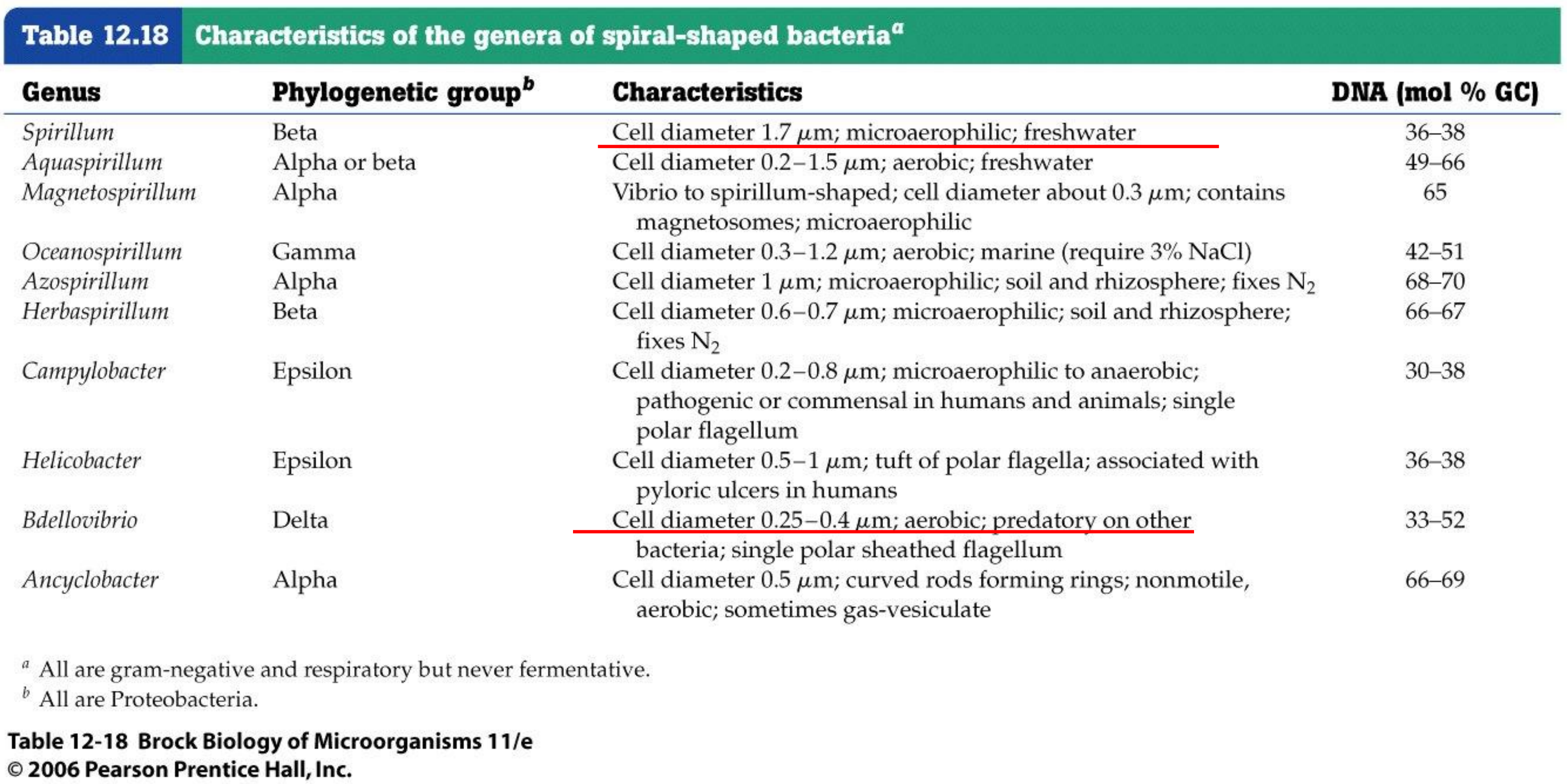

Table 12.18 Characteristics of the genera of spiral-shaped bacteria[a]

Genus	Phylogenetic group[b]	Characteristics	DNA (mol % GC)
Spirillum	Beta	Cell diameter 1.7 μm; microaerophilic; freshwater	36–38
Aquaspirillum	Alpha or beta	Cell diameter 0.2–1.5 μm; aerobic; freshwater	49–66
Magnetospirillum	Alpha	Vibrio to spirillum-shaped; cell diameter about 0.3 μm; contains magnetosomes; microaerophilic	65
Oceanospirillum	Gamma	Cell diameter 0.3–1.2 μm; aerobic; marine (require 3% NaCl)	42–51
Azospirillum	Alpha	Cell diameter 1 μm; microaerophilic; soil and rhizosphere; fixes N_2	68–70
Herbaspirillum	Beta	Cell diameter 0.6–0.7 μm; microaerophilic; soil and rhizosphere; fixes N_2	66–67
Campylobacter	Epsilon	Cell diameter 0.2–0.8 μm; microaerophilic to anaerobic; pathogenic or commensal in humans and animals; single polar flagellum	30–38
Helicobacter	Epsilon	Cell diameter 0.5–1 μm; tuft of polar flagella; associated with pyloric ulcers in humans	36–38
Bdellovibrio	Delta	Cell diameter 0.25–0.4 μm; aerobic; predatory on other bacteria; single polar sheathed flagellum	33–52
Ancyclobacter	Alpha	Cell diameter 0.5 μm; curved rods forming rings; nonmotile, aerobic; sometimes gas-vesiculate	66–69

[a] All are gram-negative and respiratory but never fermentative.

[b] All are Proteobacteria.

Table 12-18 Brock Biology of Microorganisms 11/e

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Bdellovibrio – бактерия - внутриклеточный паразит других бактерий

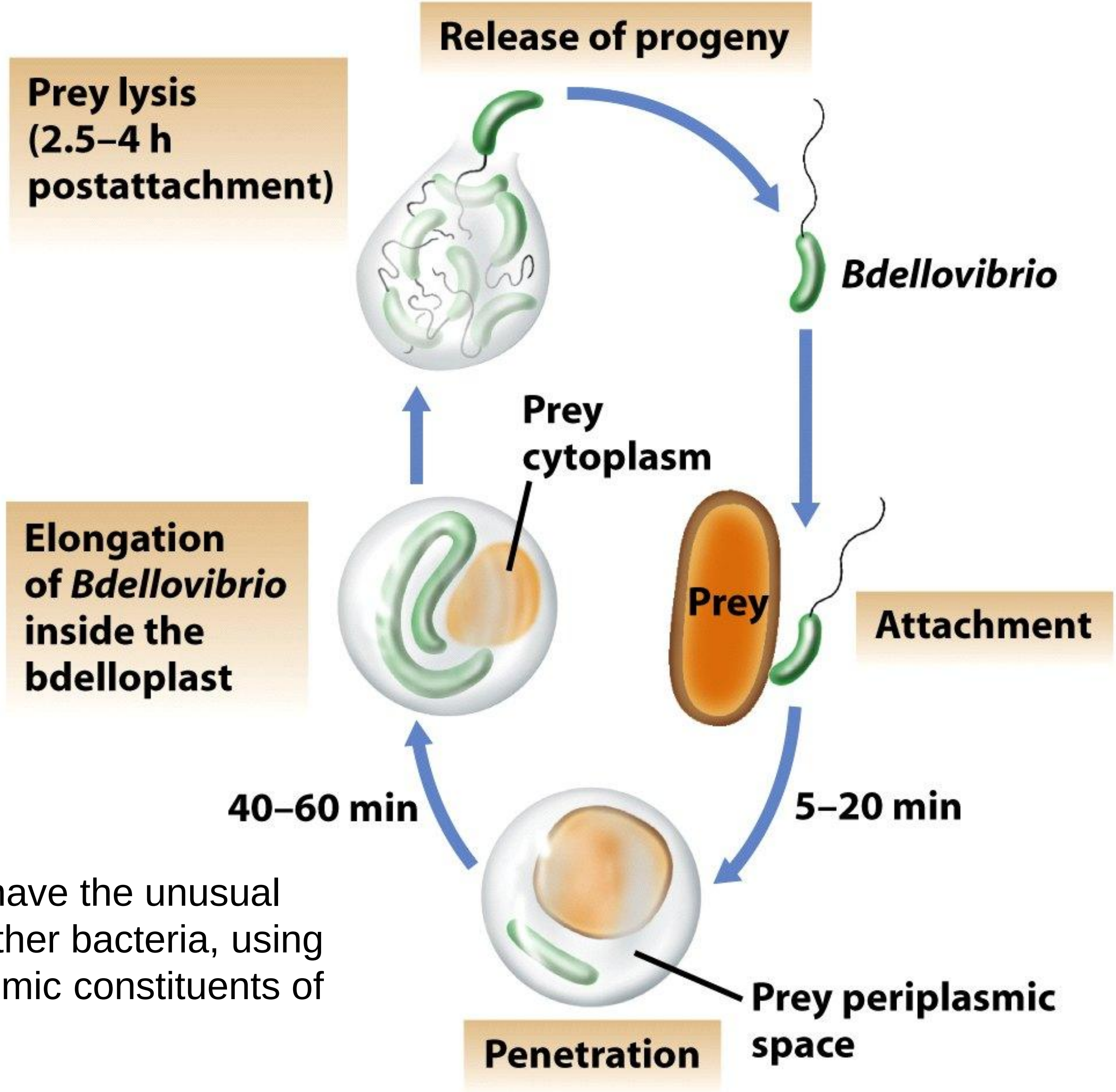

Figure 12-34b Brock Biology of Microorganisms 11/e
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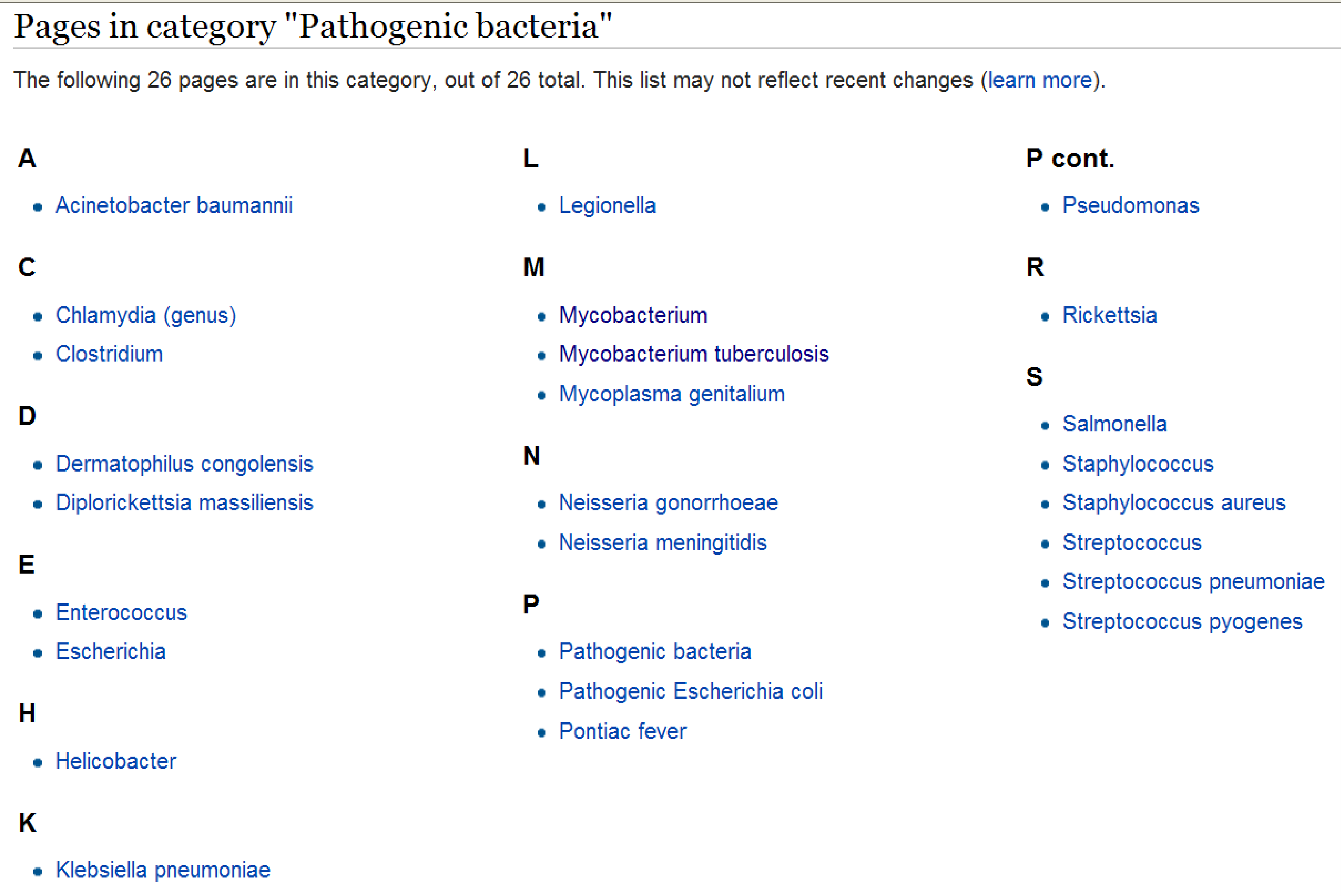
Bdellovibrio organisms have the unusual property of preying on other bacteria, using as nutrients the cytoplasmic constituents of their hosts

Бактерии - внутриклеточные паразиты эукариот



Pages in category "Pathogenic bacteria"

The following 26 pages are in this category, out of 26 total. This list may not reflect recent changes (learn more).

A

- Acinetobacter baumannii

C

- Chlamydia (genus)
- Clostridium

D

- Dermatophilus congolensis
- Diplorickettsia massiliensis

E

- Enterococcus
- Escherichia

H

- Helicobacter

K

- Klebsiella pneumoniae

L

- Legionella

M

- Mycobacterium
- Mycobacterium tuberculosis
- Mycoplasma genitalium

N

- Neisseria gonorrhoeae
- Neisseria meningitidis

P

- Pathogenic bacteria
- Pathogenic Escherichia coli
- Pontiac fever

P cont.

- Pseudomonas

R

- Rickettsia

S

- Salmonella
- Staphylococcus
- Staphylococcus aureus
- Streptococcus
- Streptococcus pneumoniae
- Streptococcus pyogenes

Бактерии

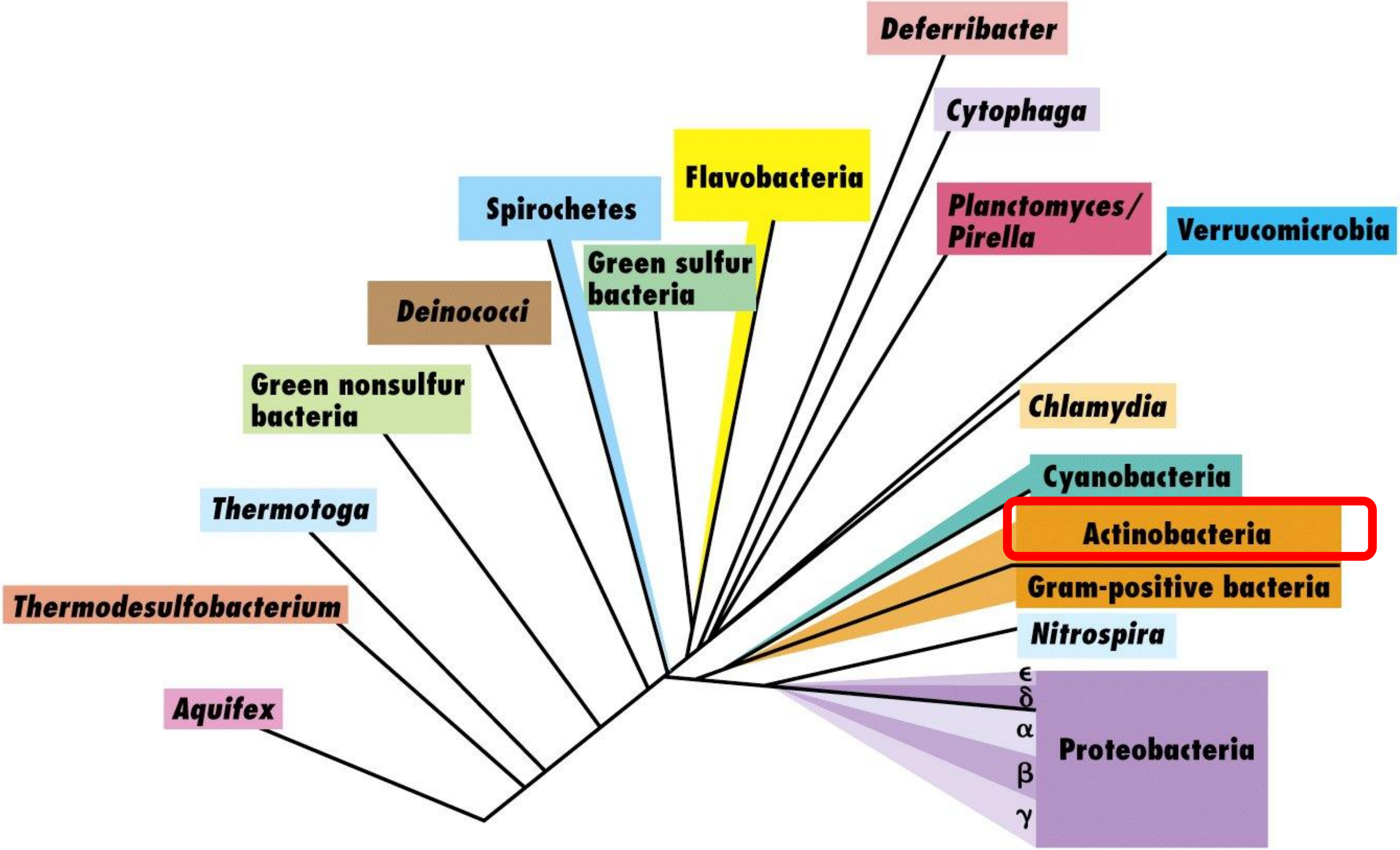


Figure 12-1 Brock Biology of Microorganisms 11/e
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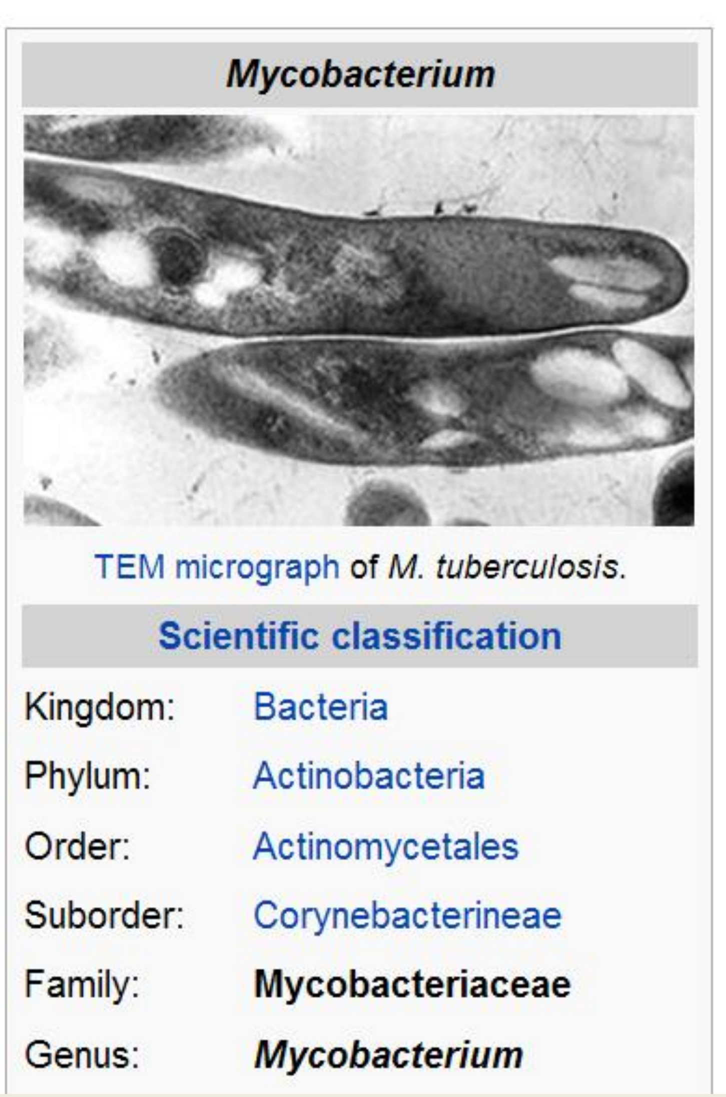
Mycobacterium is a genus of Actinobacteria. The genus includes pathogens known to cause serious diseases in mammals, including tuberculosis (*Mycobacterium tuberculosis*) and leprosy (

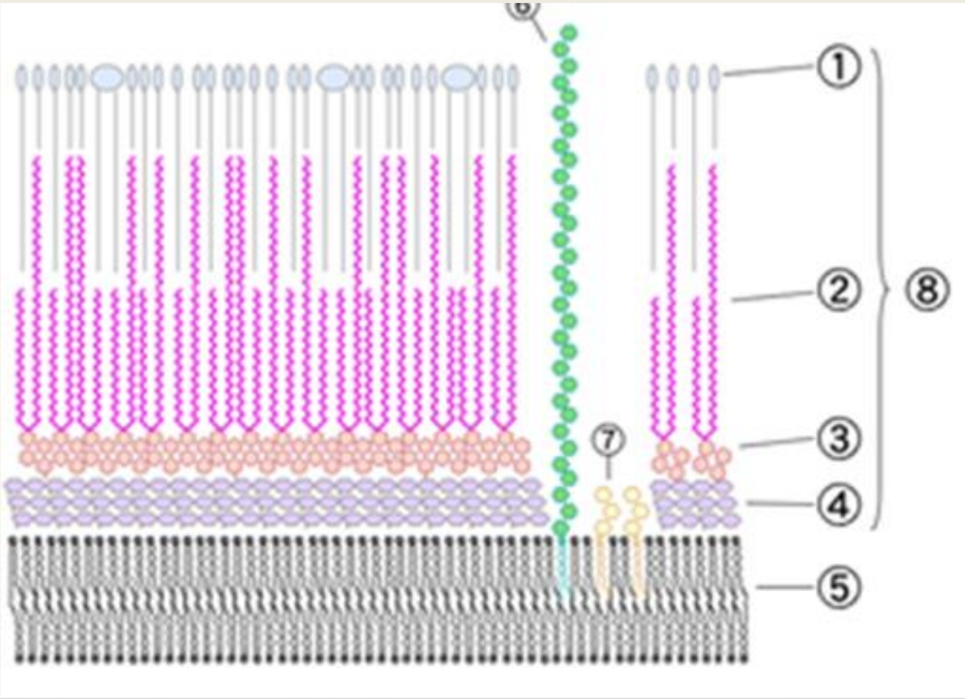


Mycobacterium

TEM micrograph of *M. tuberculosis*.

Scientific classification

Kingdom:	Bacteria
Phylum:	Actinobacteria
Order:	Actinomycetales
Suborder:	Corynebacterineae
Family:	**Mycobacteriaceae**
Genus:	***Mycobacterium***



ycobacterial cell wall: 1-outer lipids, 2-mycolic acid, 3-polysaccharides (arabinogalactan), 4-eptidoglycan, 5-plasma membrane, 6-ipoarabinomannan (LAM), 7-phosphatidylinositol nannoside, 8-cell wall skeleton

Бактерии

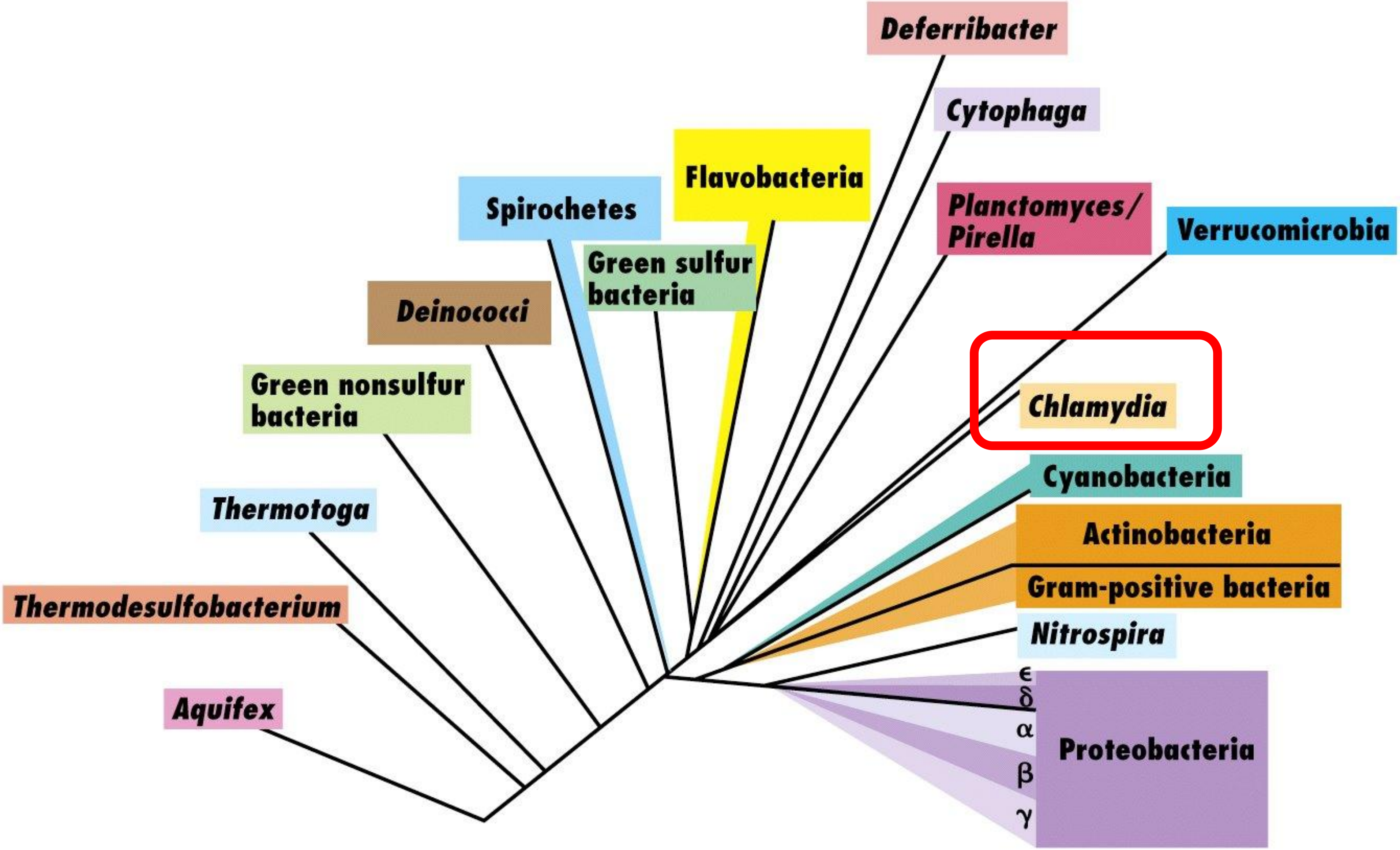


Figure 12-1 Brock Biology of Microorganisms 11/e

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Chlamydia

Obligate intracellular organisms.

Chlamydias **contain a very small genome** and are apparently deficient in many metabolic functions.

- Contain DNA and RNA. Possess ribosomes, synthesize proteins, nucleic acid, and lipids, **but cannot synthesize ATP.**
- Binary fission
- Susceptible to numerous antibiotics, but not to penicillin (**lack peptidoglycan, muramic acid**)

Cell wall:

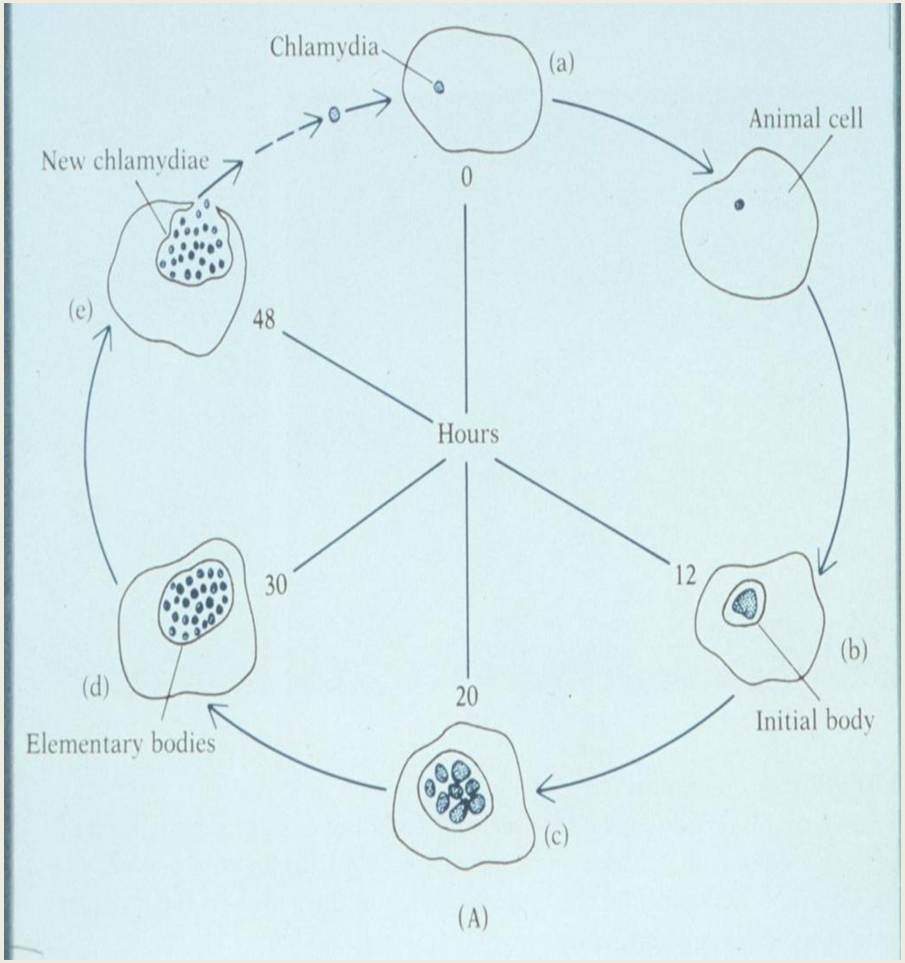
- **Major outer membrane protein (MOMP)** – serological variants (serovars)
- **Outer membrane protein 2 (OMP2)** – cysteine-rich protein, structure stability of elementary body (EB)

Unique development cycle

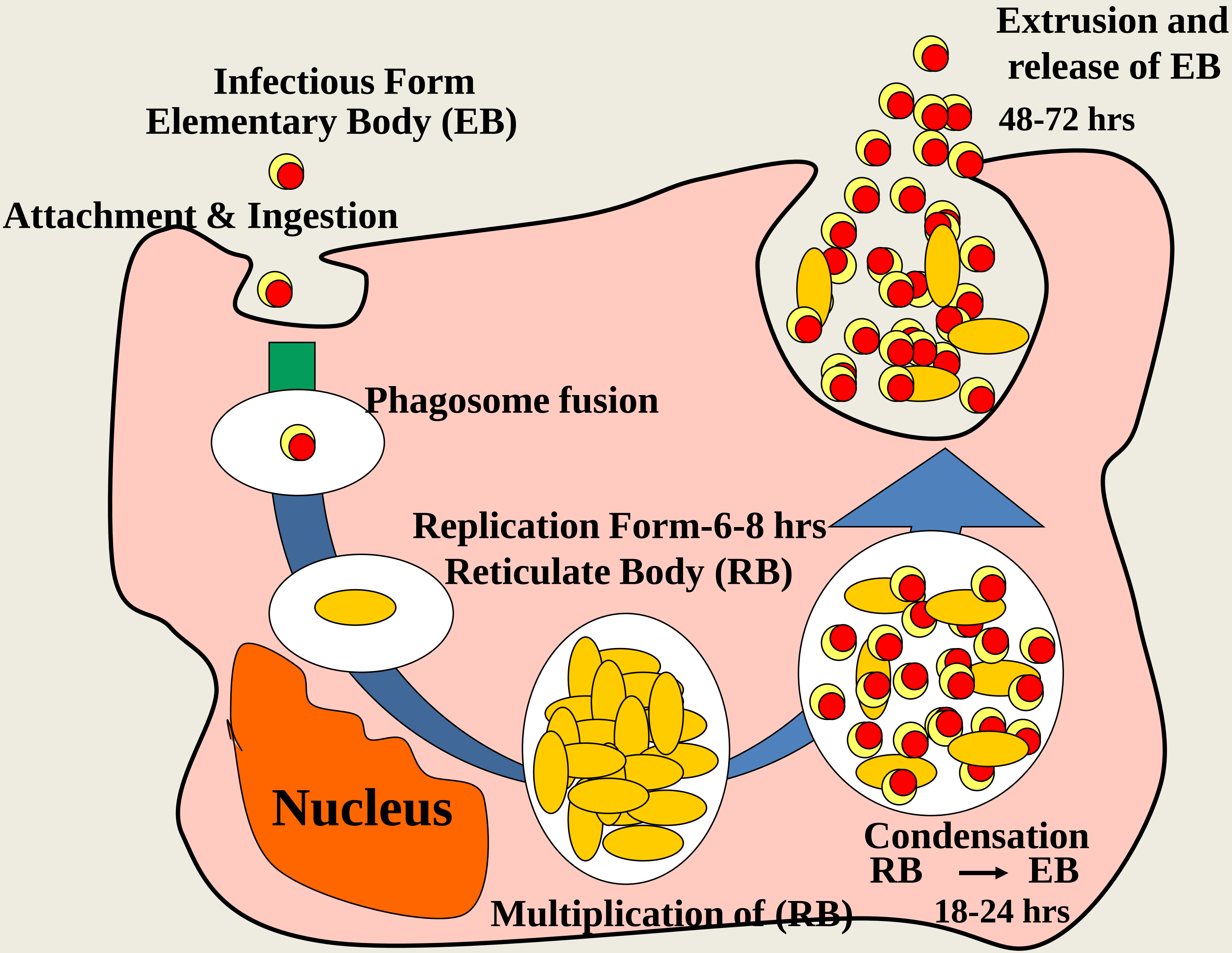
Two morphological distinct forms in cytoplasmic phagosome:

(1) **elementary body** (300-400 nm), resistant to harsh environmental factors; bind to receptors of host cells and stimulate uptake; cannot replicate but **infectious**,

(2) **reticulate body** (800-1000 nm), reproductive form, metabolically active, **noninfectious**.



Life cycle of *C. trachomatis*



Infectious Form
Elementary Body (EB)

Attachment & Ingestion

Phagosome fusion

Replication Form-6-8 hrs
Reticulate Body (RB)

Multiplication of (RB)

Condensation
RB ⟶ EB
18-24 hrs

Extrusion and
release of EB
48-72 hrs

Nucleus

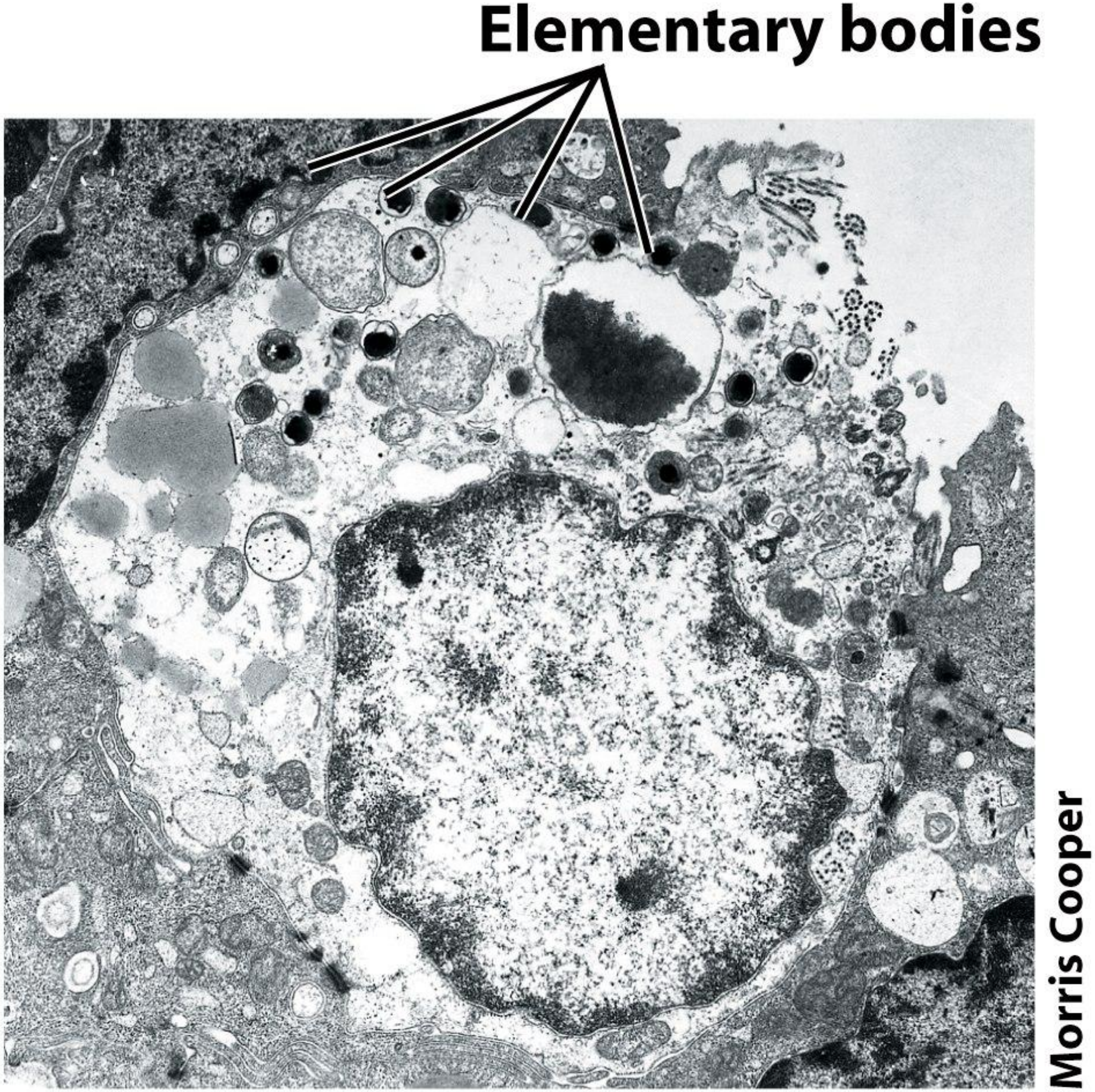


Figure 12-85b Brock Biology of Microorganisms 11/e

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Бактерии

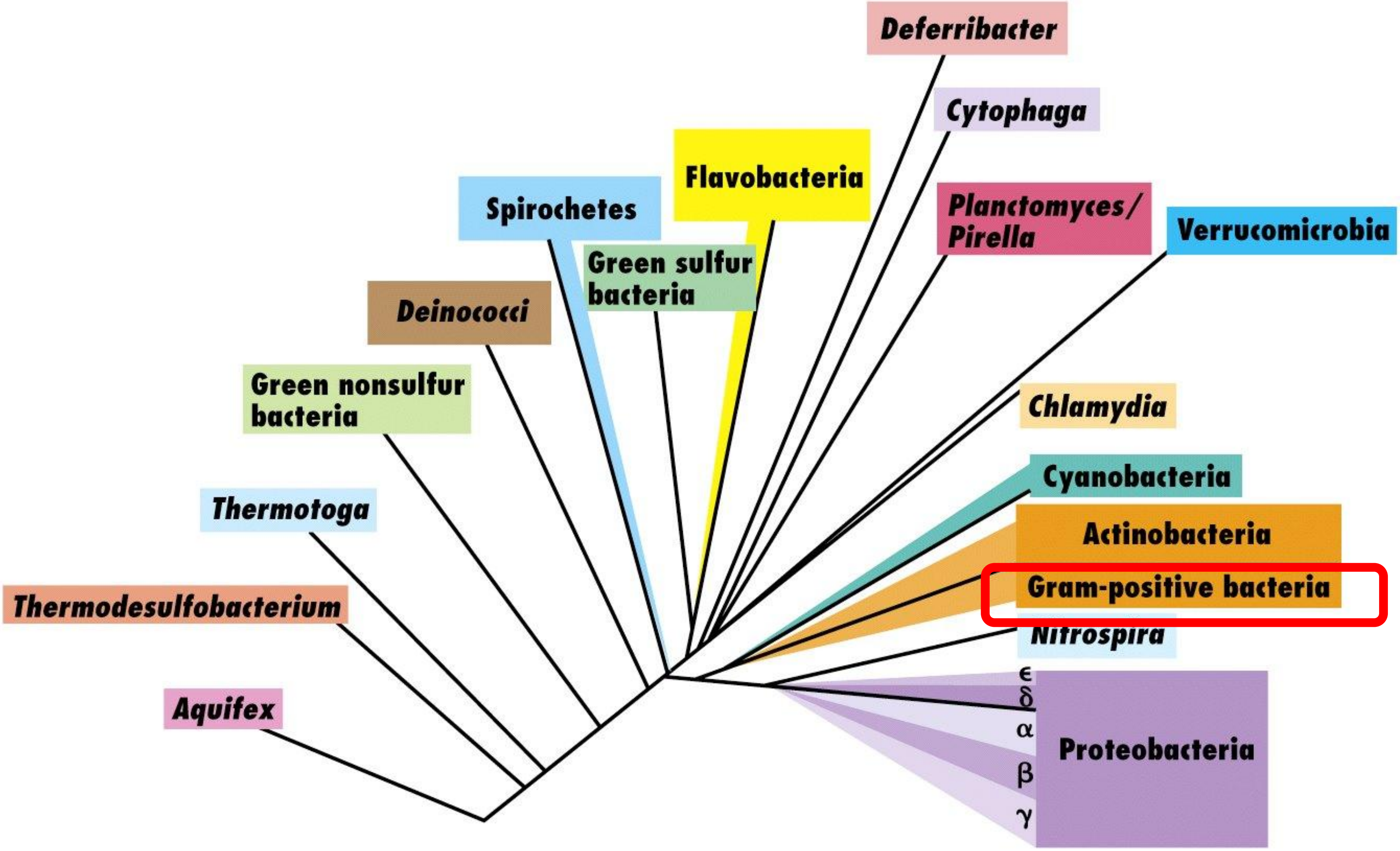

Figure 12-1 Brock Biology of Microorganisms 11/e

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Mollicute genera

Mycoplasma	~100 species; pathogens & commensals

Not related to known bacteria closest to Streptococci

Ureaplasma	pathogens & commensals
Spiroplasma	plant pathogens; insect commensal (? pathogens)
Anaeroplasma	rumen commensal
Acholeplasma	mainly commensal

Mollicutes - The smallest cells capable of self-replication

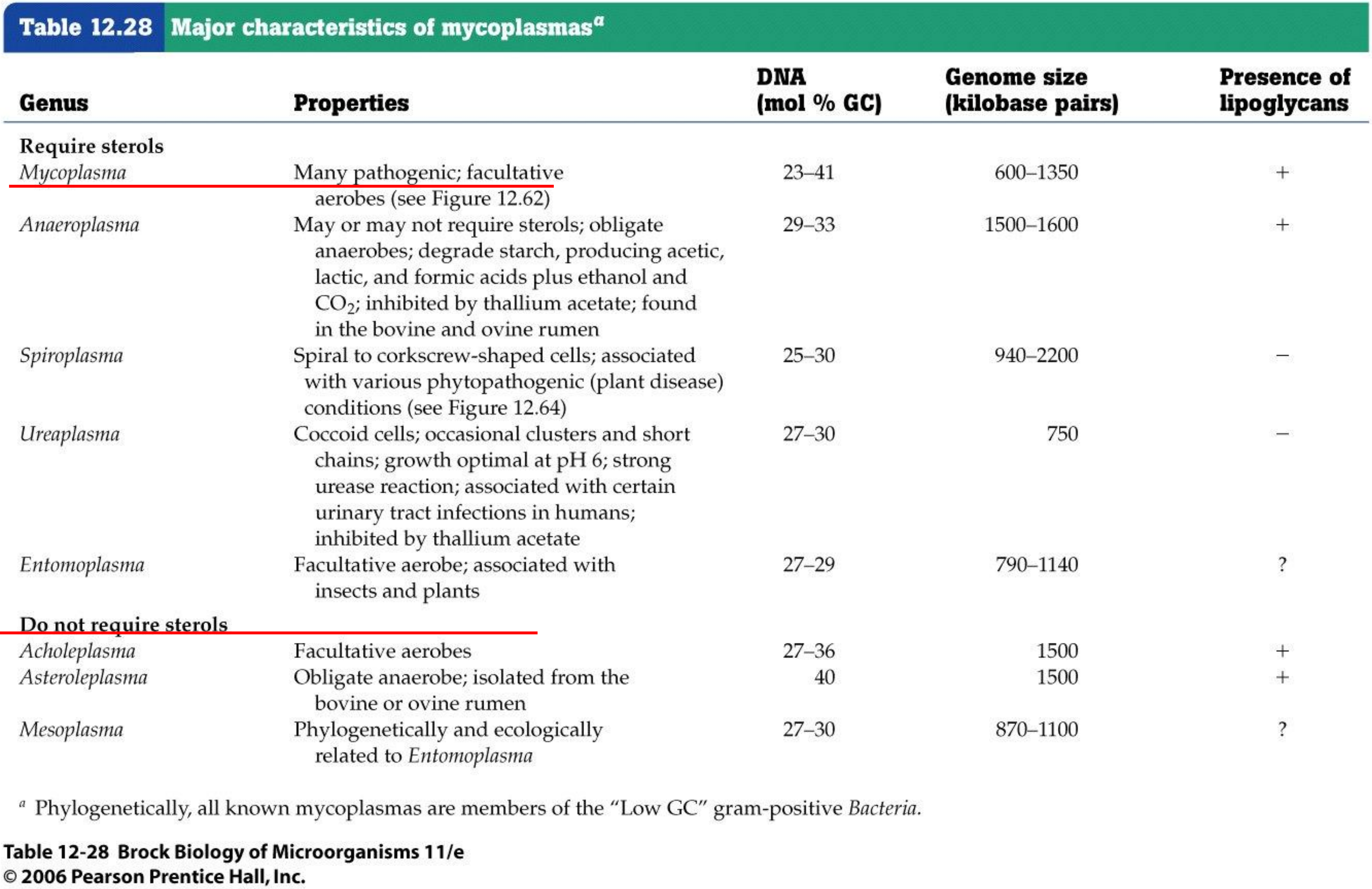

Table 12.28 Major characteristics of mycoplasmas[a]

Genus	Properties	DNA (mol % GC)	Genome size (kilobase pairs)	Presence of lipoglycans
Require sterols				
Mycoplasma	Many pathogenic; facultative aerobes (see Figure 12.62)	23–41	600–1350	+
Anaeroplasma	May or may not require sterols; obligate anaerobes; degrade starch, producing acetic, lactic, and formic acids plus ethanol and CO_2; inhibited by thallium acetate; found in the bovine and ovine rumen	29–33	1500–1600	+
Spiroplasma	Spiral to corkscrew-shaped cells; associated with various phytopathogenic (plant disease) conditions (see Figure 12.64)	25–30	940–2200	−
Ureaplasma	Coccoid cells; occasional clusters and short chains; growth optimal at pH 6; strong urease reaction; associated with certain urinary tract infections in humans; inhibited by thallium acetate	27–30	750	−
Entomoplasma	Facultative aerobe; associated with insects and plants	27–29	790–1140	?
Do not require sterols				
Acholeplasma	Facultative aerobes	27–36	1500	+
Asteroleplasma	Obligate anaerobe; isolated from the bovine or ovine rumen	40	1500	+
Mesoplasma	Phylogenetically and ecologically related to *Entomoplasma*	27–30	870–1100	?

[a] Phylogenetically, all known mycoplasmas are members of the "Low GC" gram-positive *Bacteria*.

Table 12-28 Brock Biology of Microorganisms 11/e

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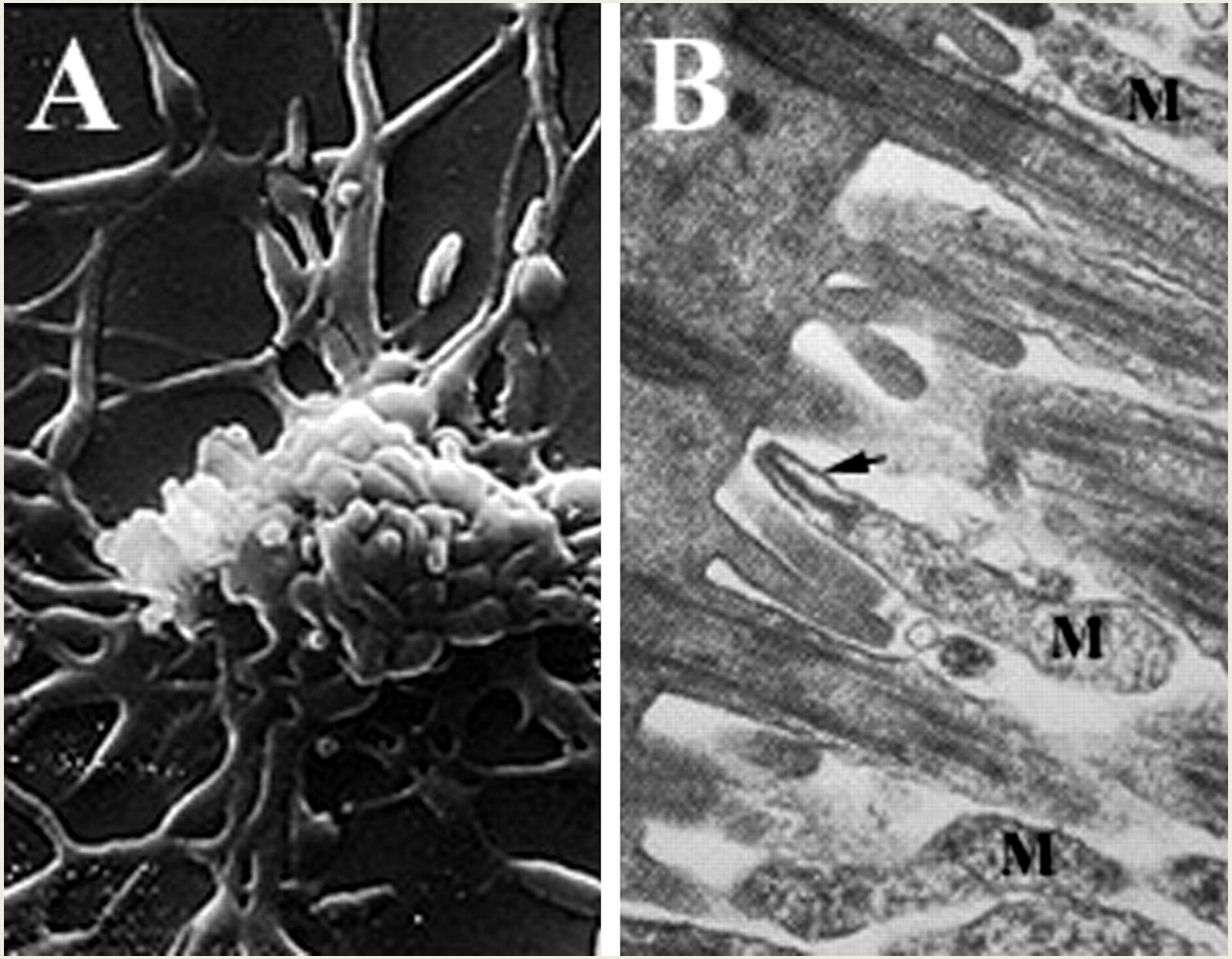
MYCOPLASMA

- 1956- PPLO (pleuropneumonia like organisms) replaced by Mycoplasma.
  - Myco : fungus like branching filaments
  - Plasma : plasticity
- highly pleomorphic – no fixed shape or size - Lack cell wall.
- Can pass through bacterial filters
- Mycoplasma cell membrane is stabilized by sterols and is resistant to lysis

Adherence of Mycoplasma pneumoniae to host cells.A: scanning electron microscopy of filamentous M. pneumoniae grown in broth medium.



Rottem S Physiol Rev 2003;83:417-432

Physiological Reviews

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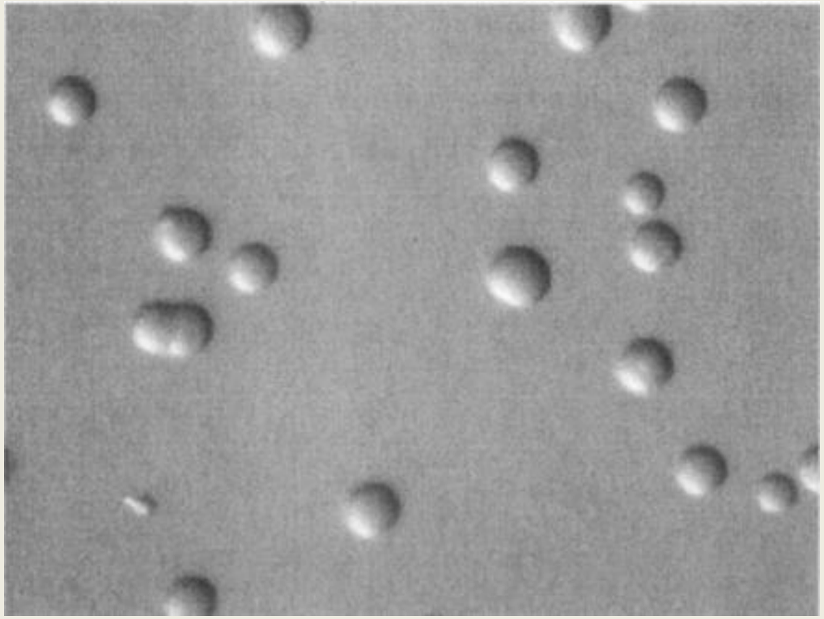
Cell Wall–Less, Low GC, Gram-Positive *Bacteria*: The Mycoplasmas

- The mycoplasma group contains organisms that lack cell walls and contain a very small genome.
- Many species require sterols to strengthen their membranes, and *several are pathogenic* for humans, other animals, and plants
- Small genome e.g. 25% of E.coli/ Genome 800 kb
- Metabolic poverty
- Not found free living
- Contaminates continuous cell cultures maintained in laboratories, Filtrable through 0.45m filters
- Interferes with the growth of viruses in these cultures.
- Mistaken for viruses.
- Eradication from infected cells is difficult.

Mycoplasma pneumoniae



Slow growing; colonies ≤ 0.1mm
(dissecting microscope)

- 1-2m x 0.1 –0.2m wide
- No cell wall; cytoskeleton maintains shape

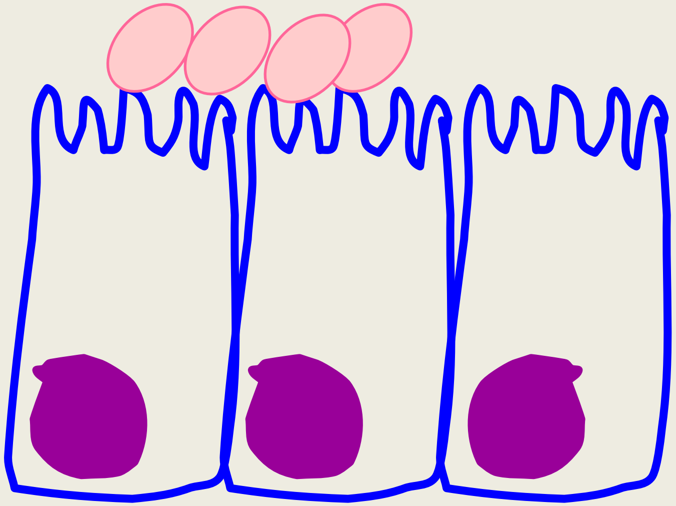
- Metabolically impoverished require serum for growth
- Grow very well in tissue culture

- **Membrane lipids similar to human cells (sterols)**

Review: M. pneumoniae…. Clin Micro Rev 2004; 17:697-728

Virulence mechanisms of mollicutes

adhesins



competition for metabolites

degradative enzymes

cytotoxic metabolites (H_2O_2; NH_4^+)

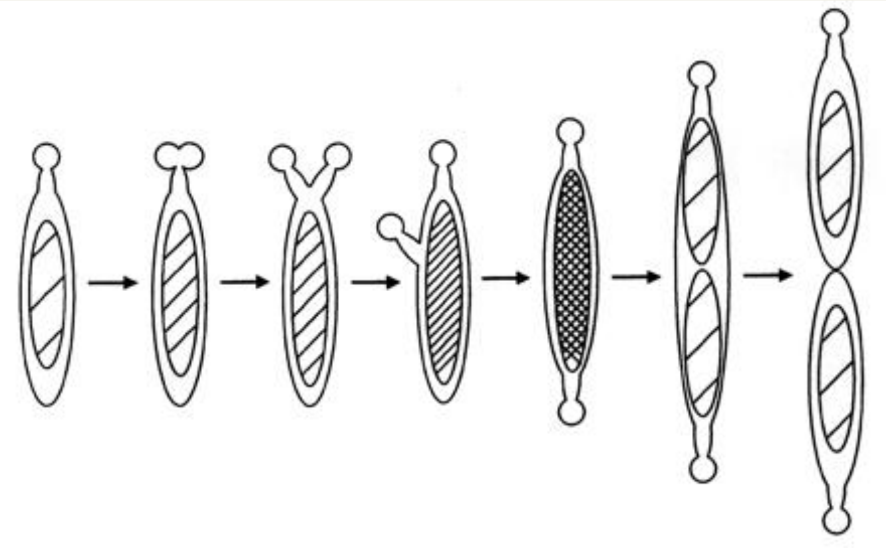
endotoxicity
(lipogalactan & lipopeptides)

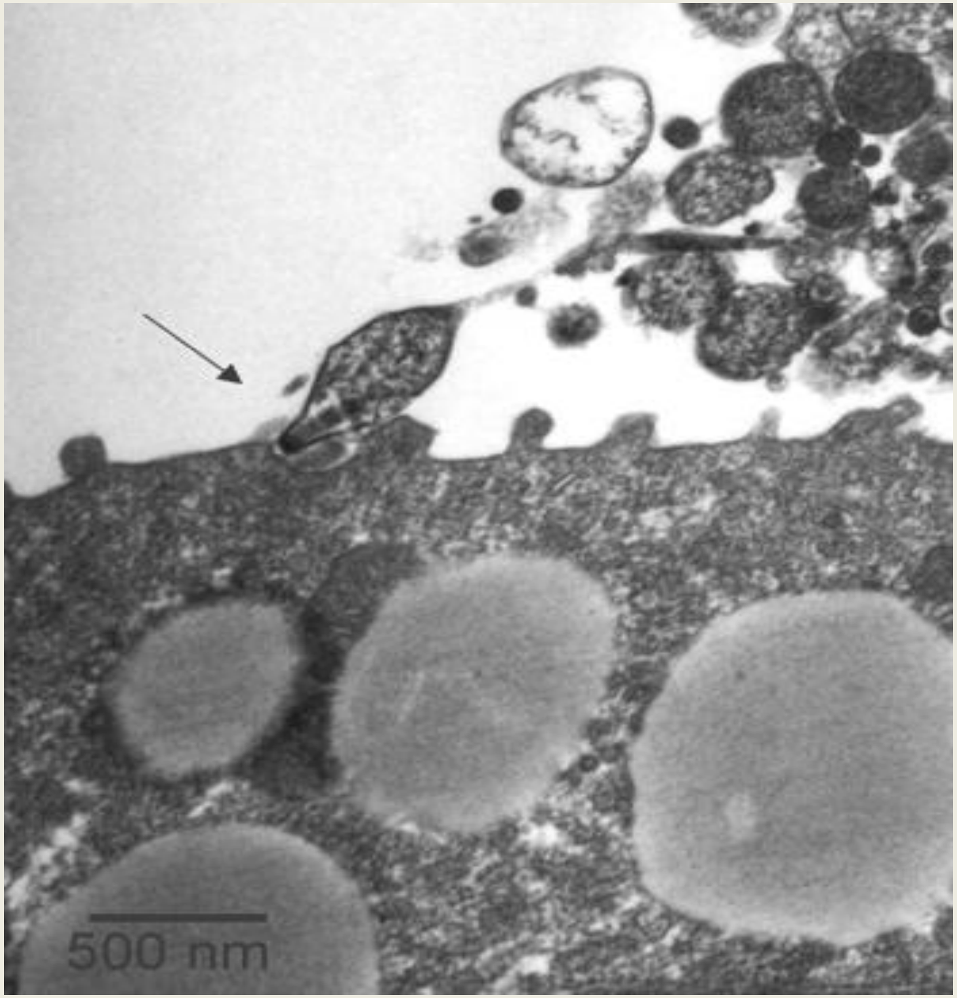
antigenic variation

intraphagocytic survival

assimilation of host cell antigens
autoimmunity
superantigenicity
antigen persistence

M. pneumoniae adherence

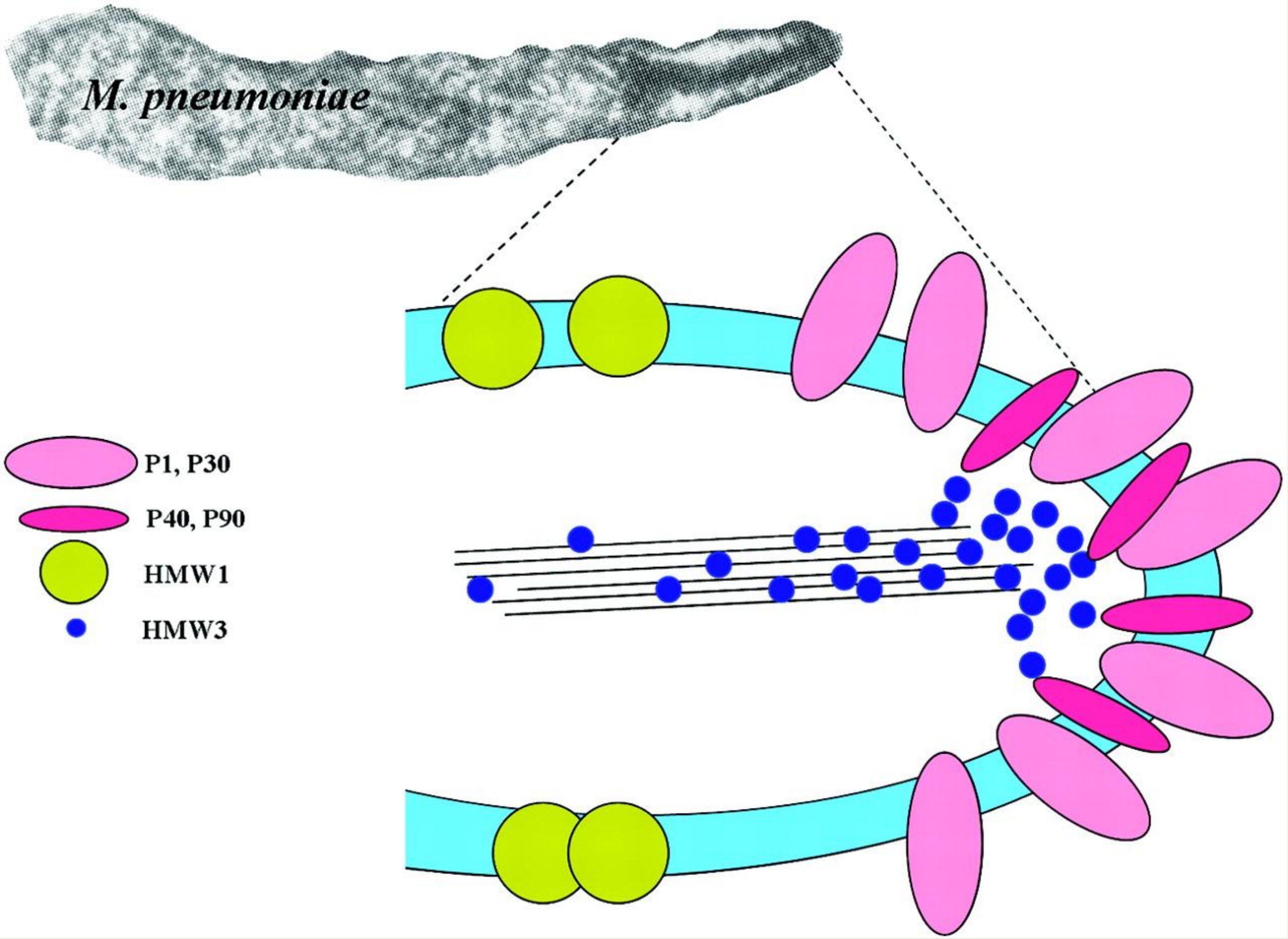




Tip organelle contains large amounts of P1 adhesin and other tip adhesins necessary for adherence to respiratory epithelium.

Other adhesins also identified

Schematic diagram of the location of the major cytadherence and accessory proteins in M. pneumoniae. [Modified from Balish and Krause (4).].



Rottem S Physiol Rev 2003;83:417-432

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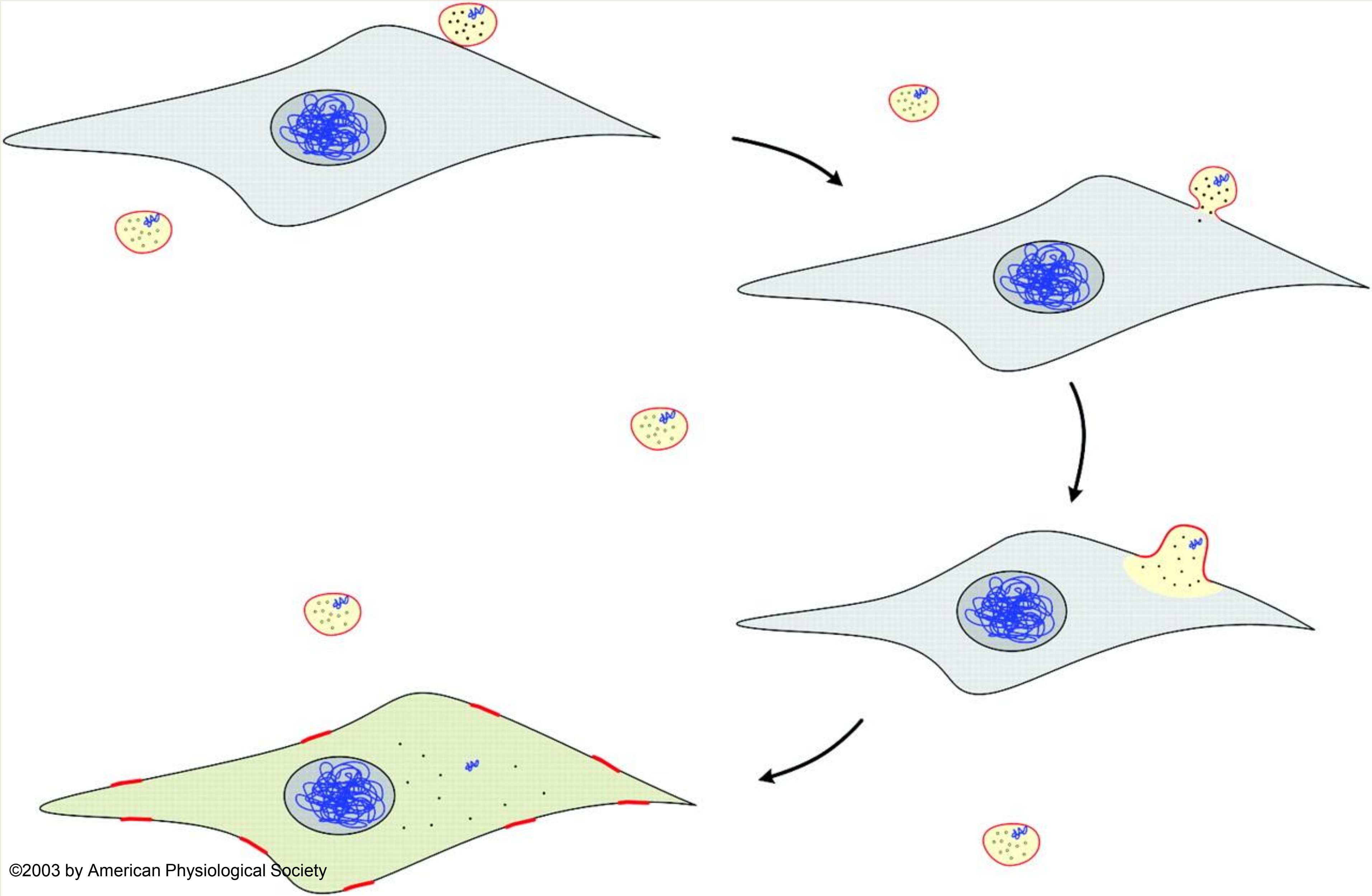
Schematic diagram of the fusion of a mycoplasma with a eukaryotic cell.

Rottem S Physiol Rev 2003;83:417-432



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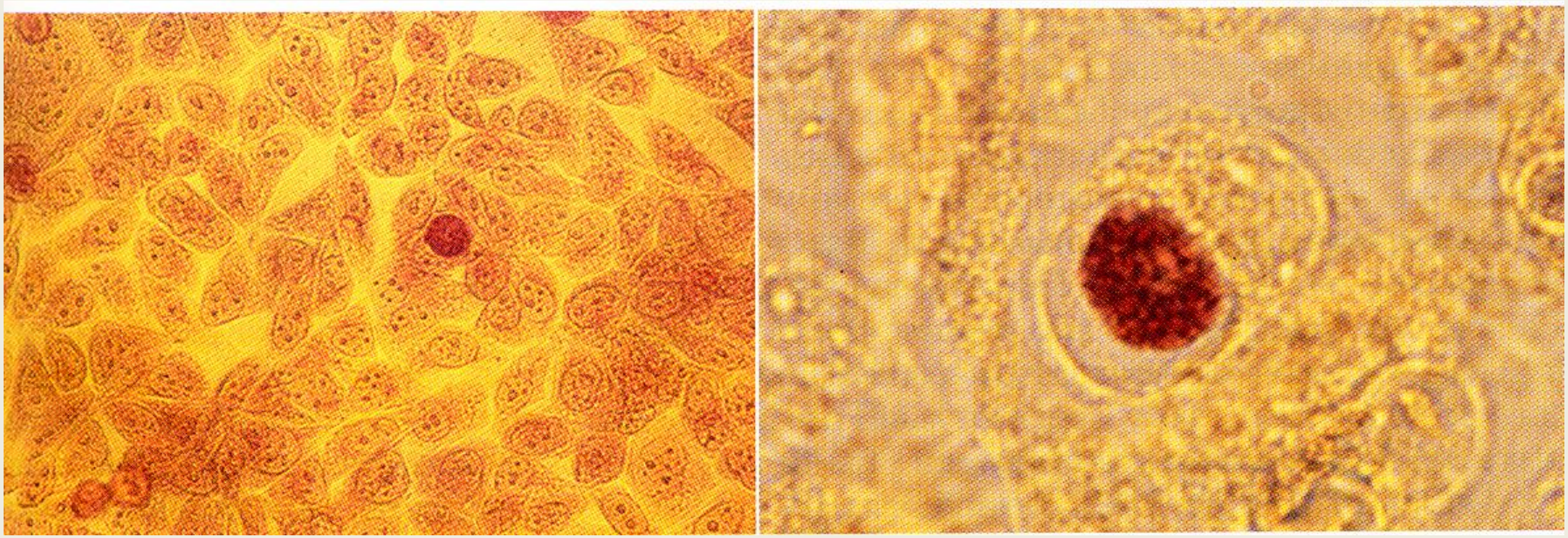
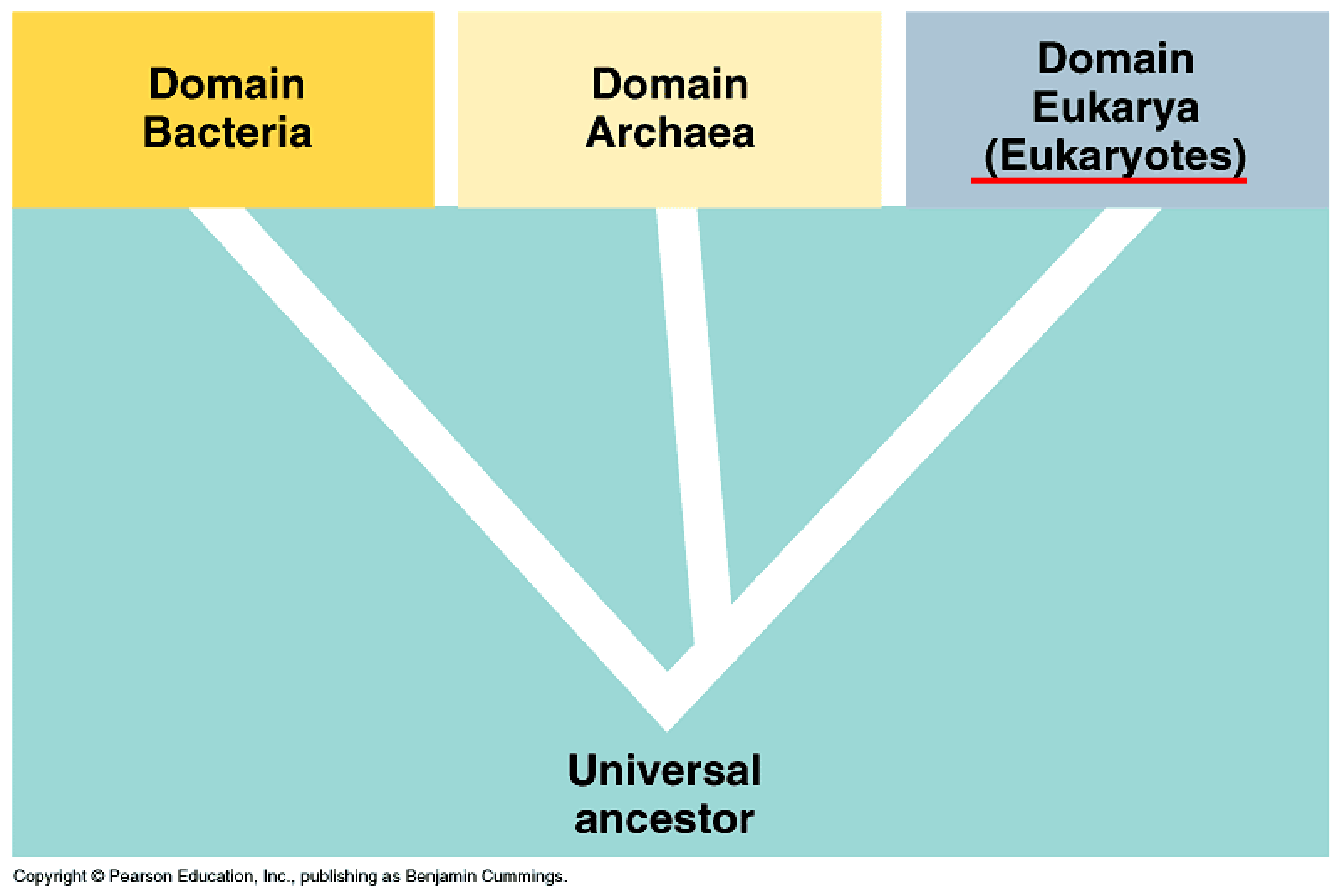
Chlamydia inclusion bodies



- Virulence factors
  - Toxicity from attachment and penetration

Простейшие (эукариоты) как внутриклеточные паразиты эукариот



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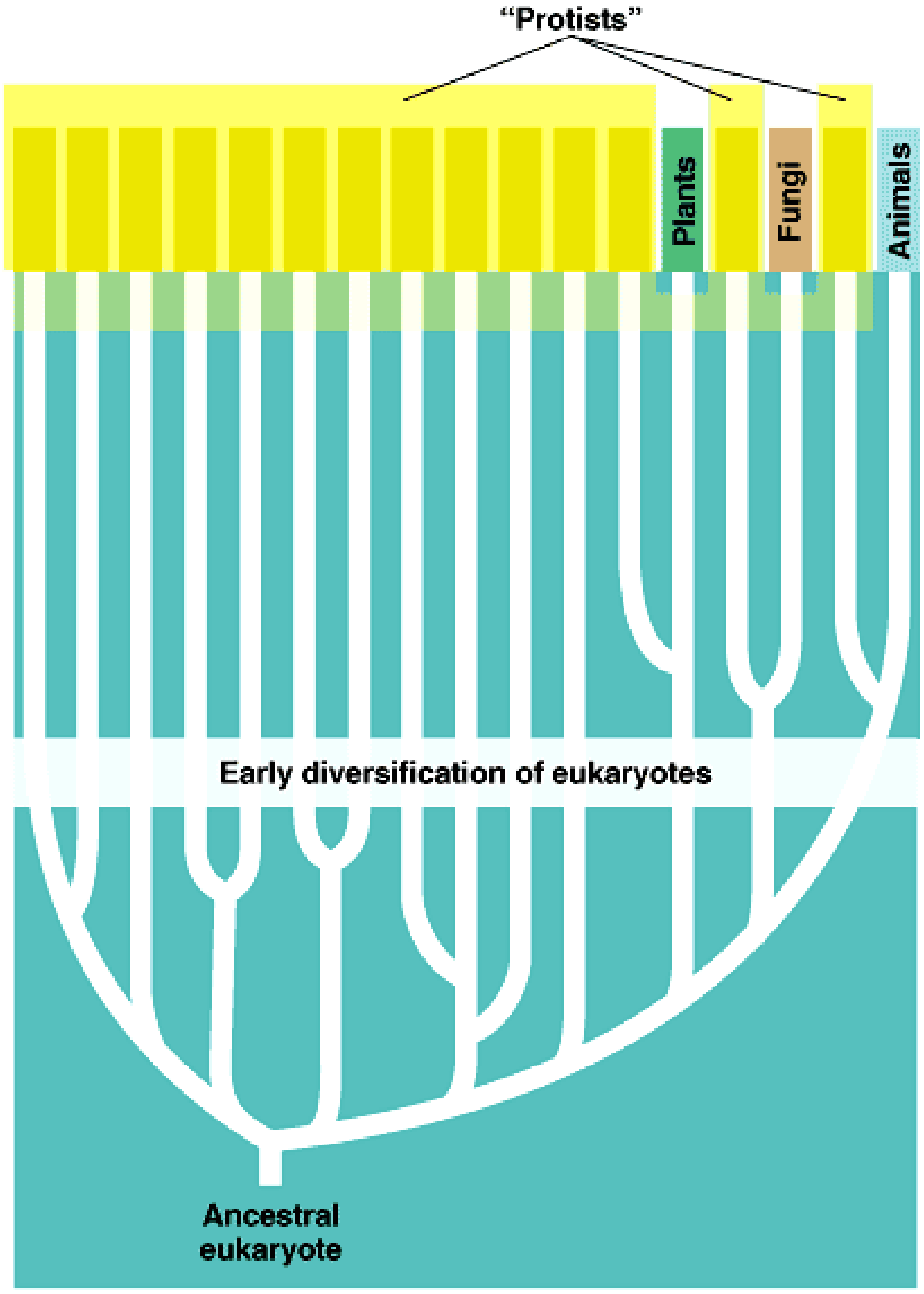
PROTISTS

Systematists split protists into many kingdoms

In the five kingdom system**, anything that wasn’t a prokaryote, plant, animal, or fungus was grouped as a protist**.

Systematists now divide protists into as many as 20 separate kingdoms.



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Life Cycle

-Most reproduce sexually by meiosis and then go on to reproduce asexually.
-Whether haploid or diploid dominates varies.
-Cysts form at some point during the life cycle of many protists. Cysts are resistant cells that are capable of surviving harsh conditions.
-There can be “alternation of generations” in many taxa.

Habitat

-Most protists are aquatic (but some in soil).
-Plankton are organisms that drift or swim near the surface of the water. Phytoplankton are responsible for half of the world’s photosynthesis and O_2 production.

Protista diversity:

Diplomonadida and Parabasala

-Lack mitochondria (probably lost them, rather than never acquired them)

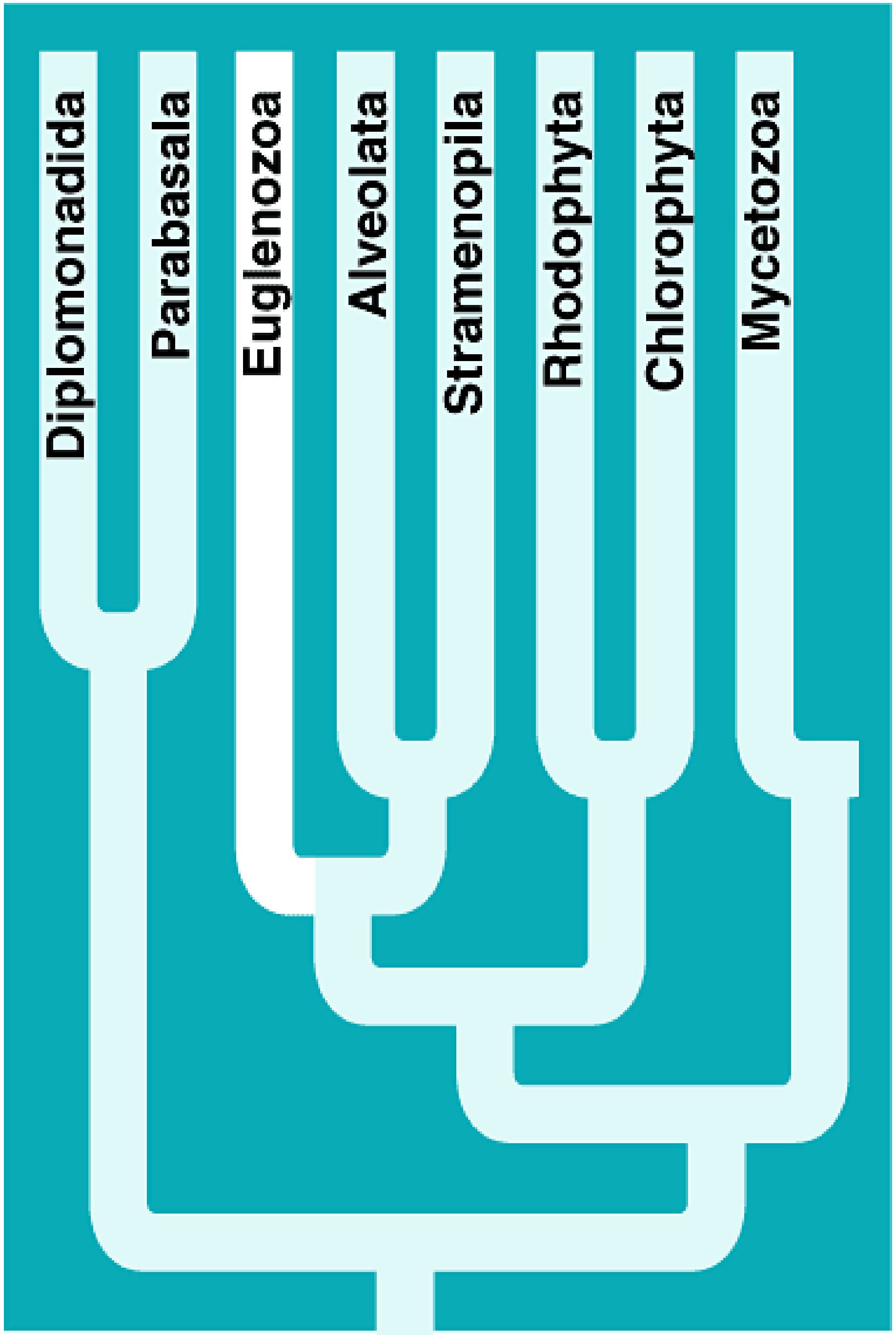
***Giardia lamblia*- example of Diplomonad -Parasite**

***Trichomonas vaginalis*- example of Parabasalid -Parasite.**

Alveolata- Apicomplexa

Apicomplexa are animal parasites with complex life cycles involving more than one host.

- *e.g.*, *Plasmodium* causes malaria.



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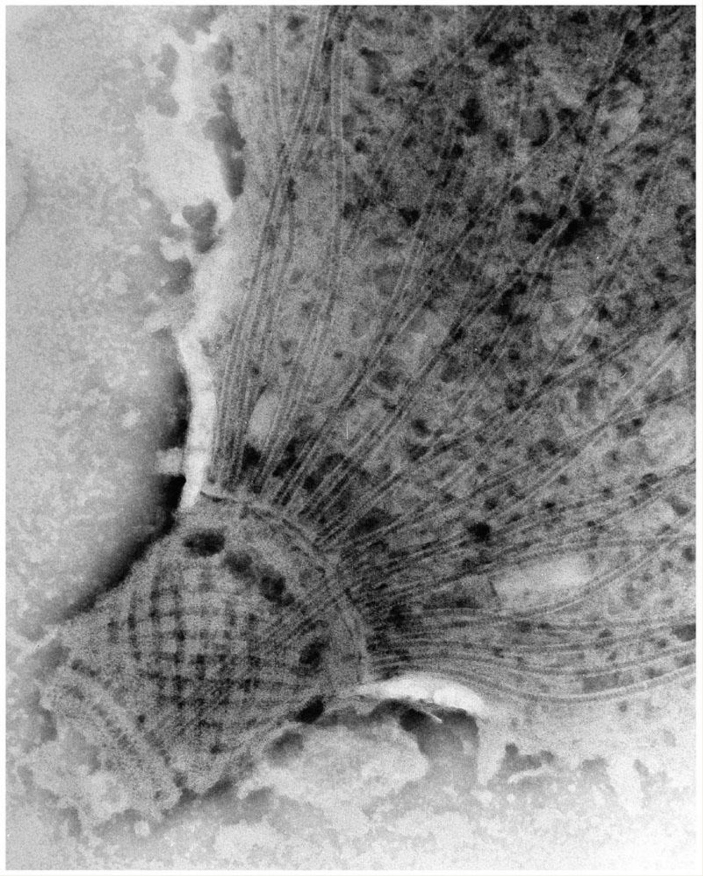
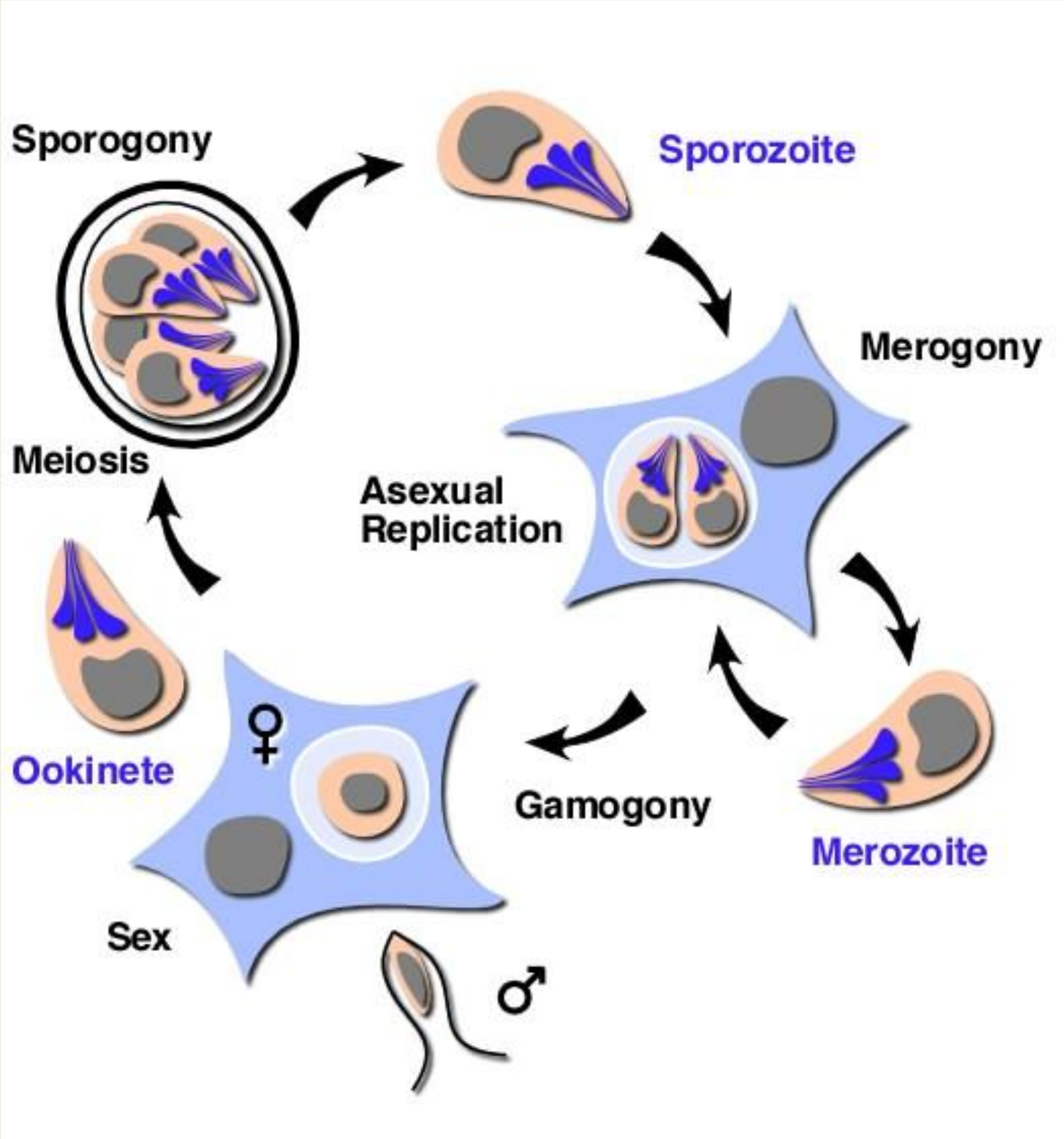
Apicomplexa



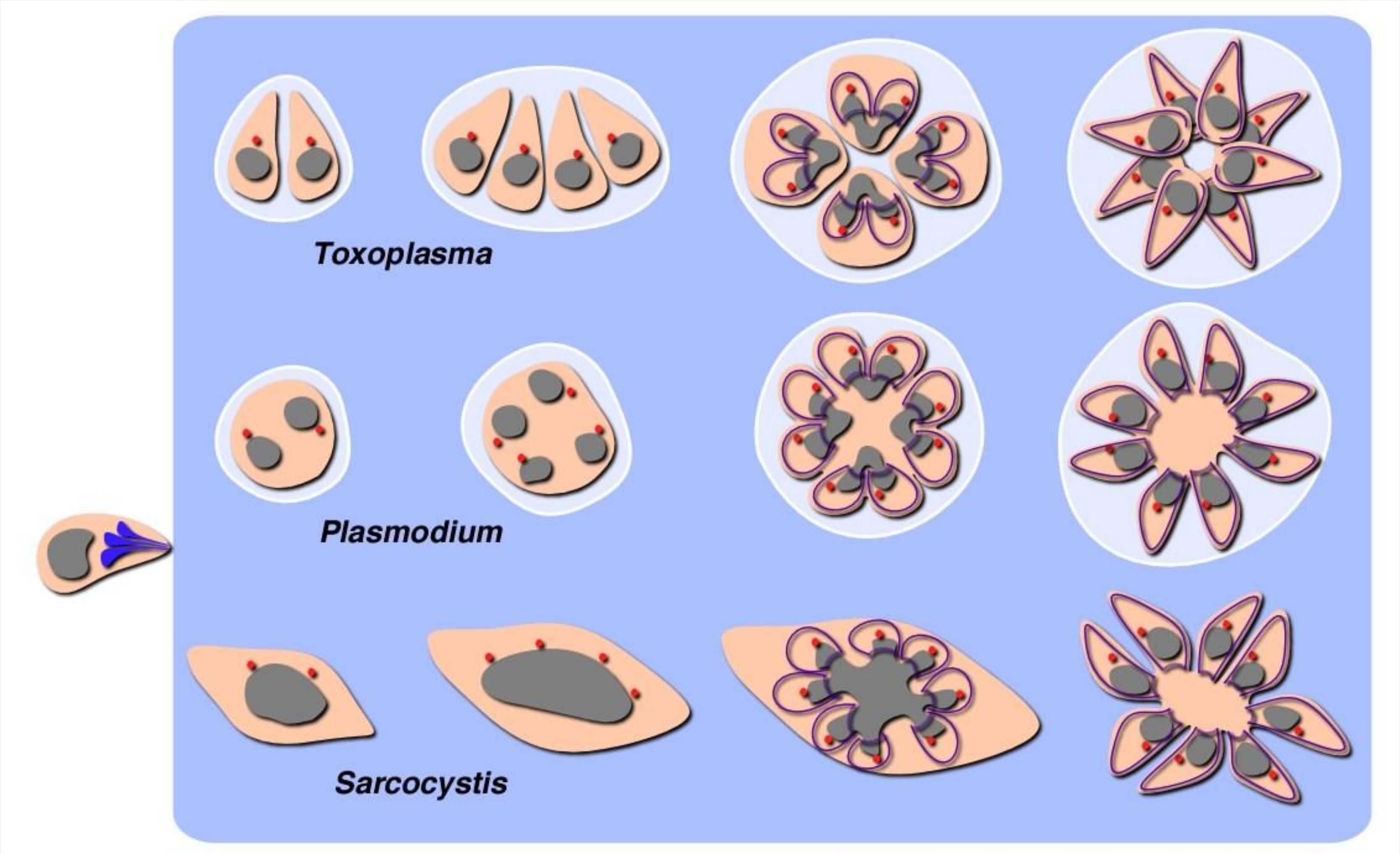
- Cells **contain an apical complex** which is an assemblage of cytoskeletal elements and secretory organelles
- No flagella or cilia except for the microgamete (sperm)
- **All members of the phylum are parasitic**

Apicomplexa

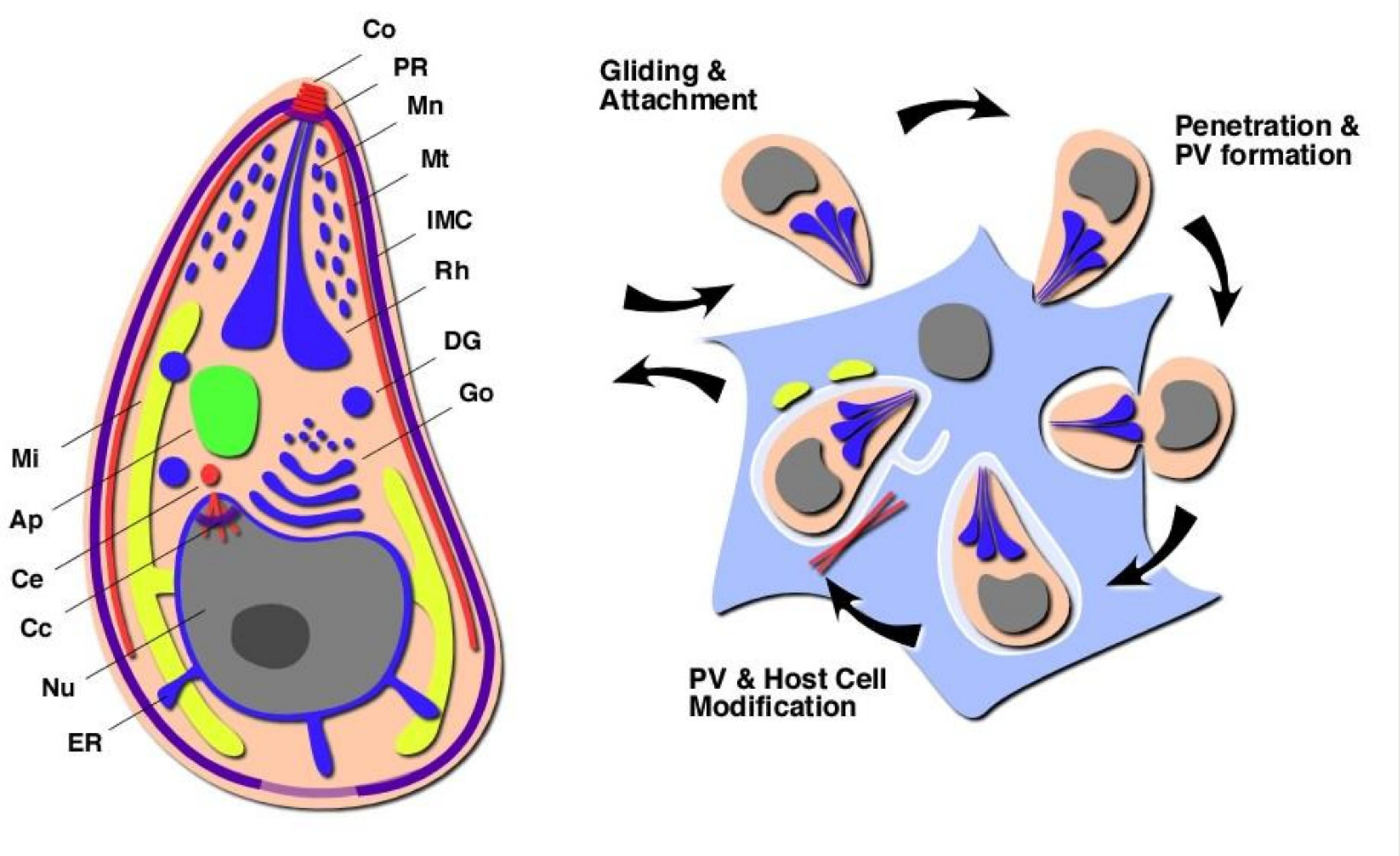


- Apicomplexans are haplonts and meiosis directly follows fertilization
- **All replication occurs inside of host cells** (with the exception of the conclusion of meiosis in certain species)
- There are several invasive zoite stages

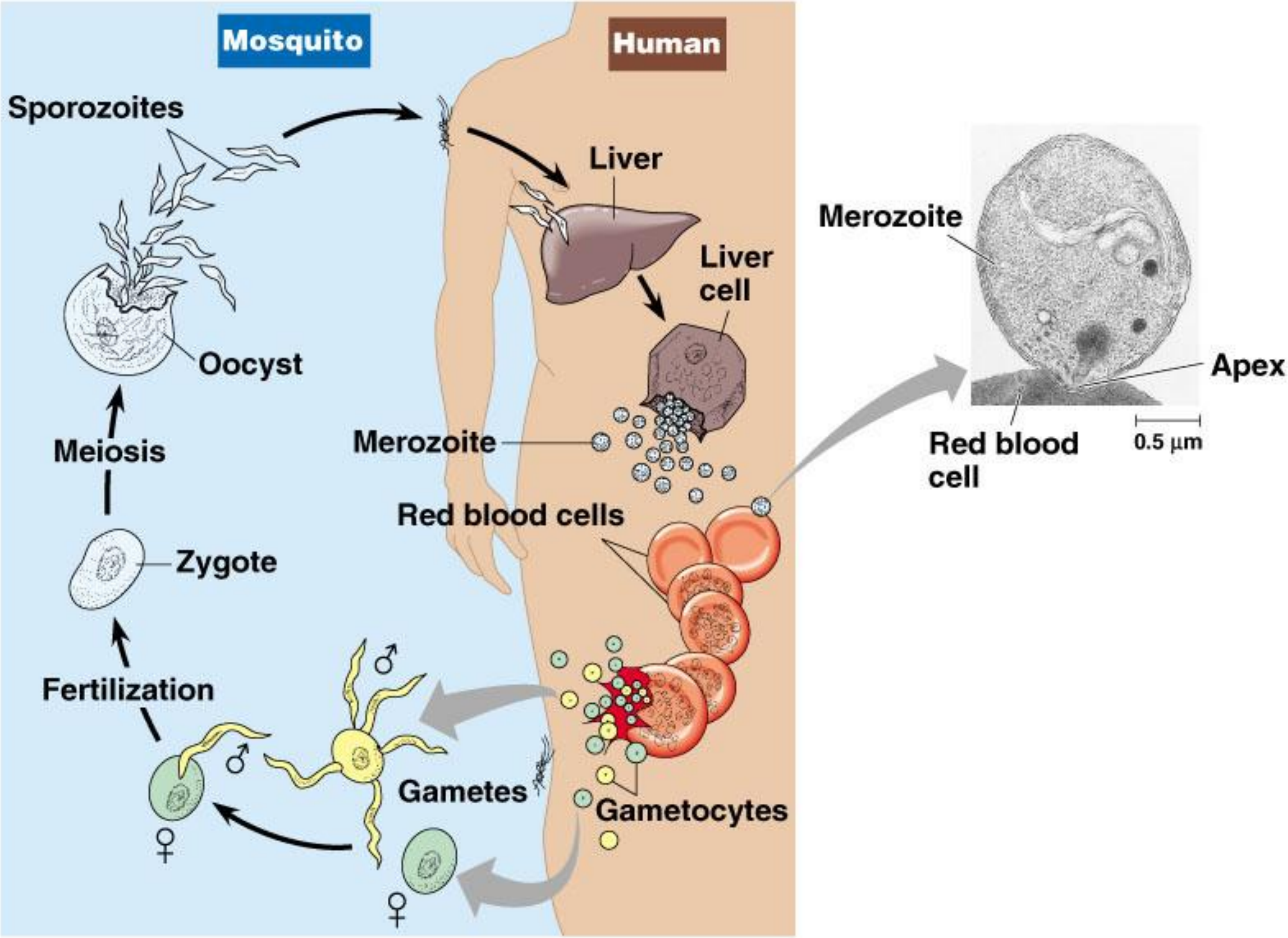
Three modes of intracellular development & replication



Apicomplexan host cell invasion



PV is the nascent parasitophorous vacuole



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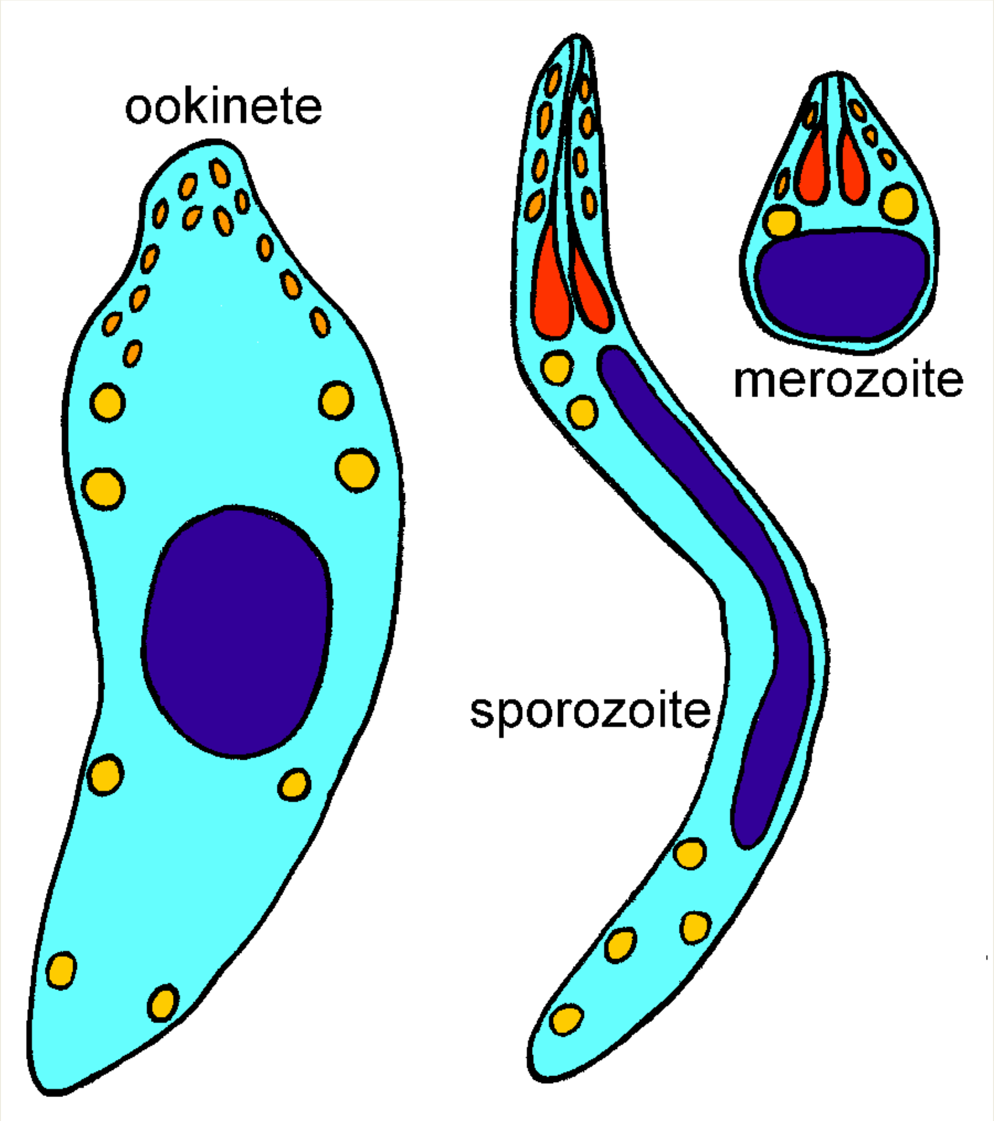
Plasmodium Invasive Stages



ookinete (motile)

- **mosquito gut epithelial cells**

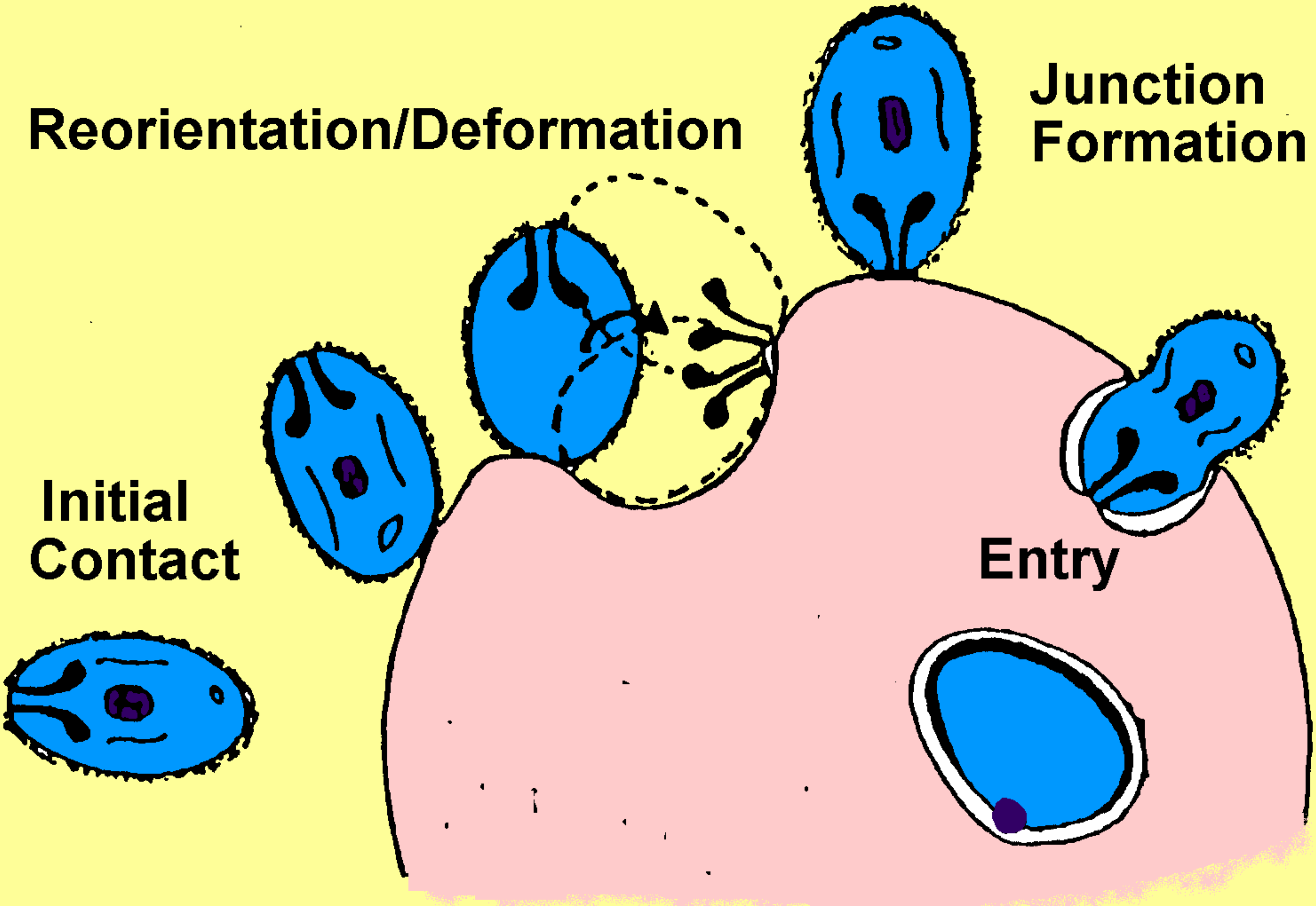
sporozoite (motile)

- **mosquito salivary glands**
- **hepatocytes**

merozoite (non-motile)

- **erythrocytes**

Steps in Merozoite Invasion

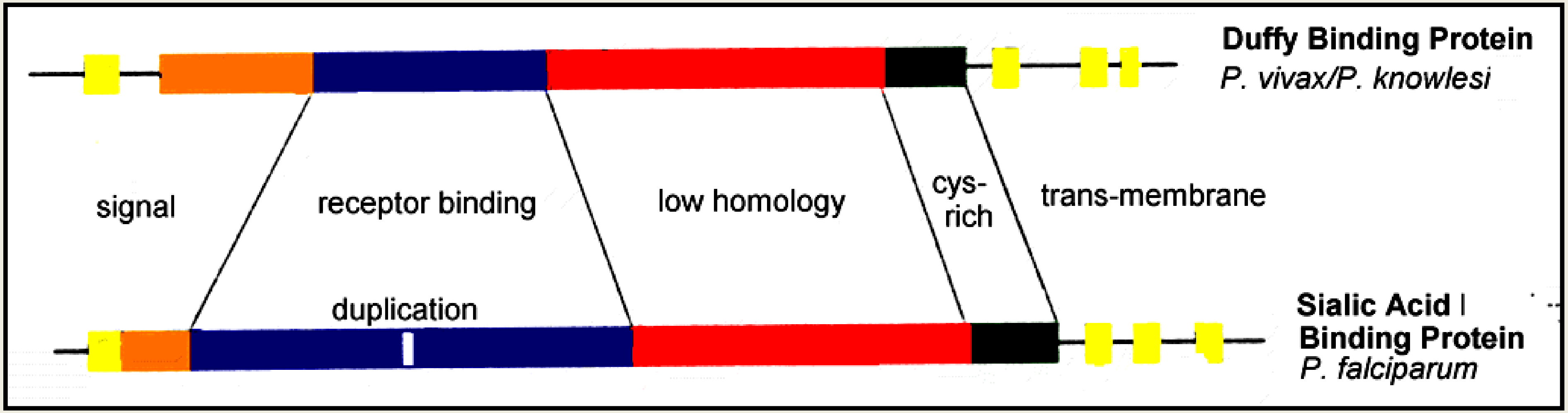


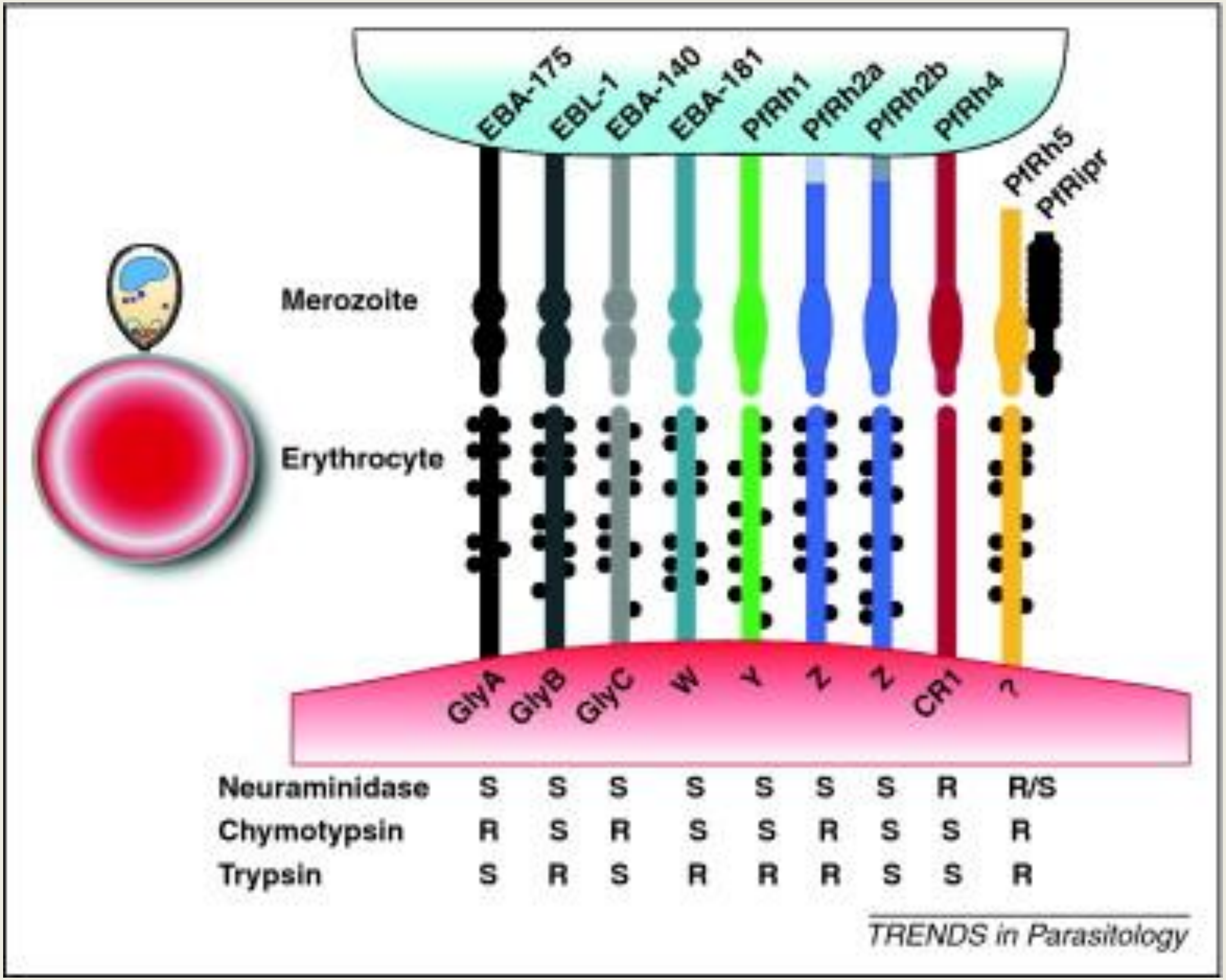
Plasmodium Merozoite invasion: a complex and ordered process

- Initial Binding
  - merozoite surface proteins (eg. MSP-1)
- Reorientation (AMA-1)
- Microneme Discharge and Junction Formation
  - Ca^{2+} signal?
  - receptor-ligand interactions (adhesive proteins)
- Rhoptry Discharge and Vacuole Formation
  - clearing of host membrane proteins
  - moving junction formation (RON2/AMA-1)
- Parasite Entry
  - mediated by actin-myosin ‘glidosome’
- Closure of PVM and Erythrocyte Membrane

Invasion Receptors/Ligands

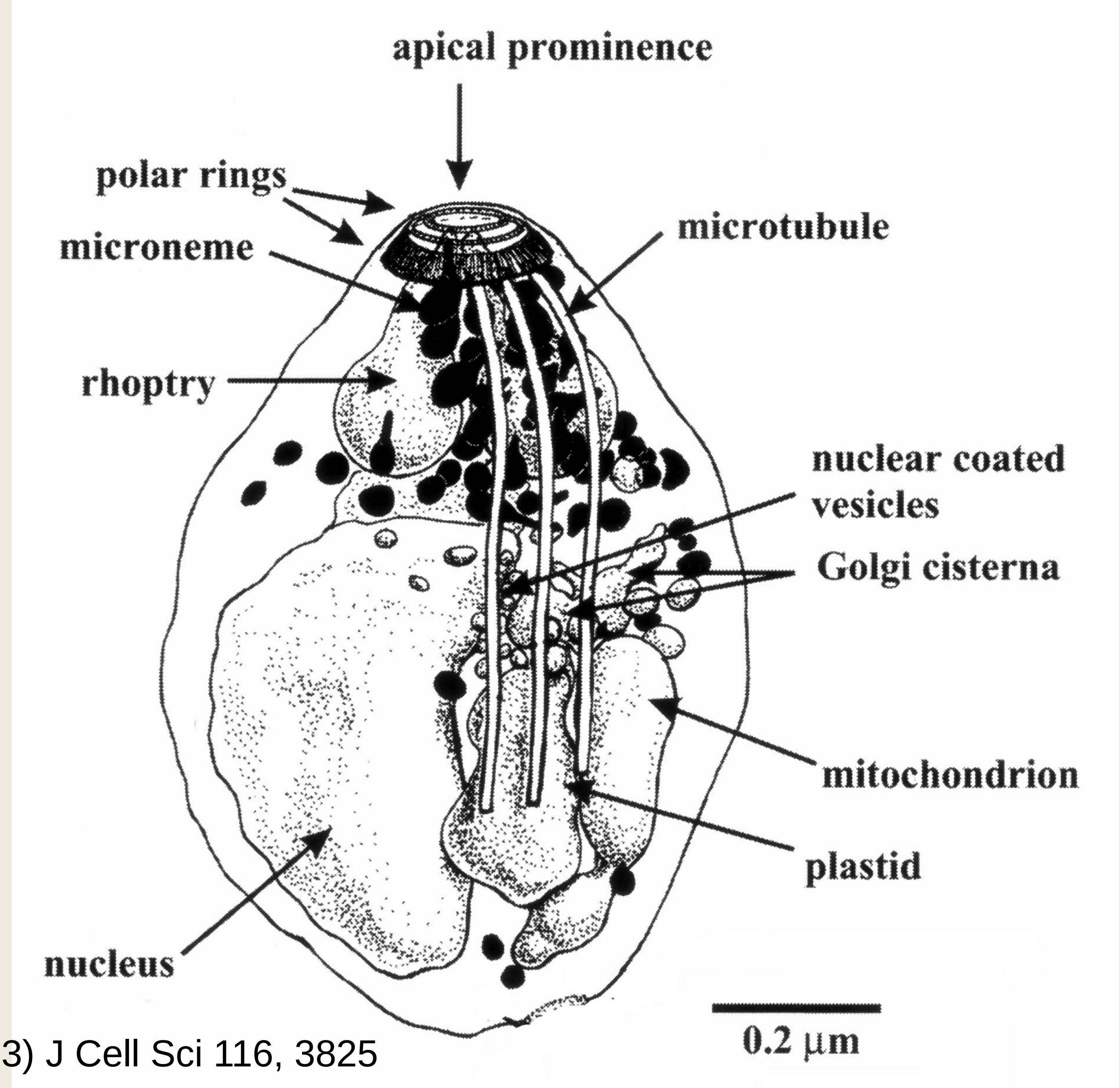
Species	Host Receptor	Merozoite Ligand
P. falciparum	glycophorins (sialic acid)	EBA-175
P. vivax, *P. knowlesi*	Duffy Ag	DBP





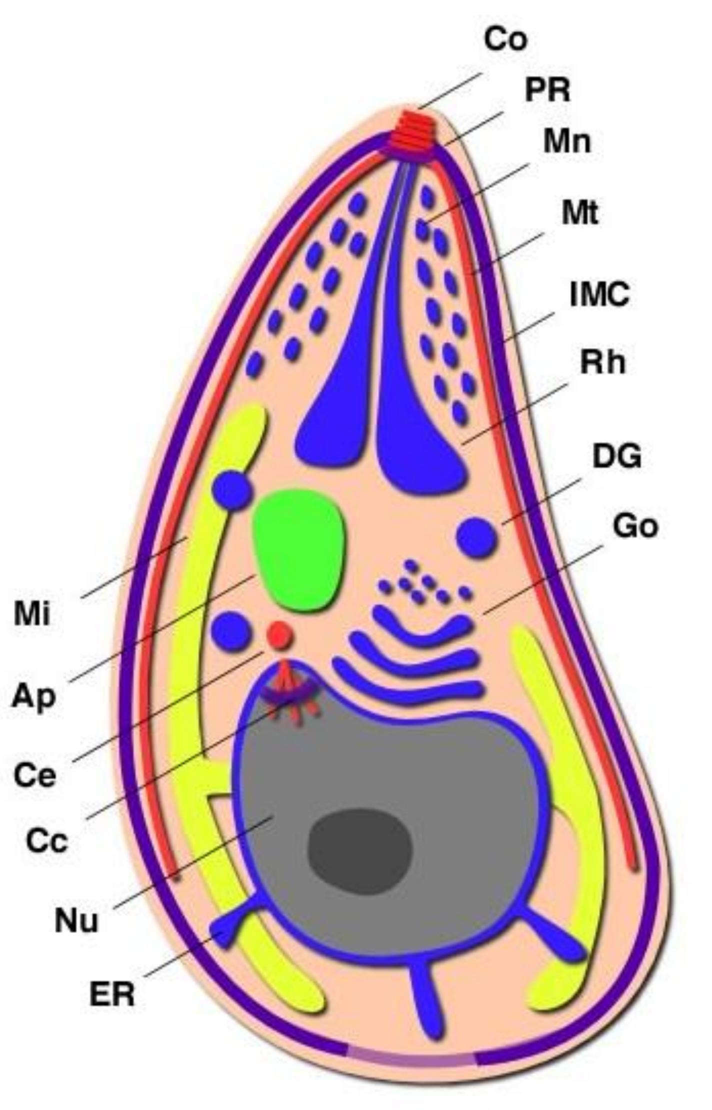
Neuraminidase	S	S	S	S	S	S	S	R	R/S
Chymotypsin	R	S	R	S	S	R	S	S	R
Trypsin	S	R	S	R	R	R	S	S	R

TRENDS in Parasitology



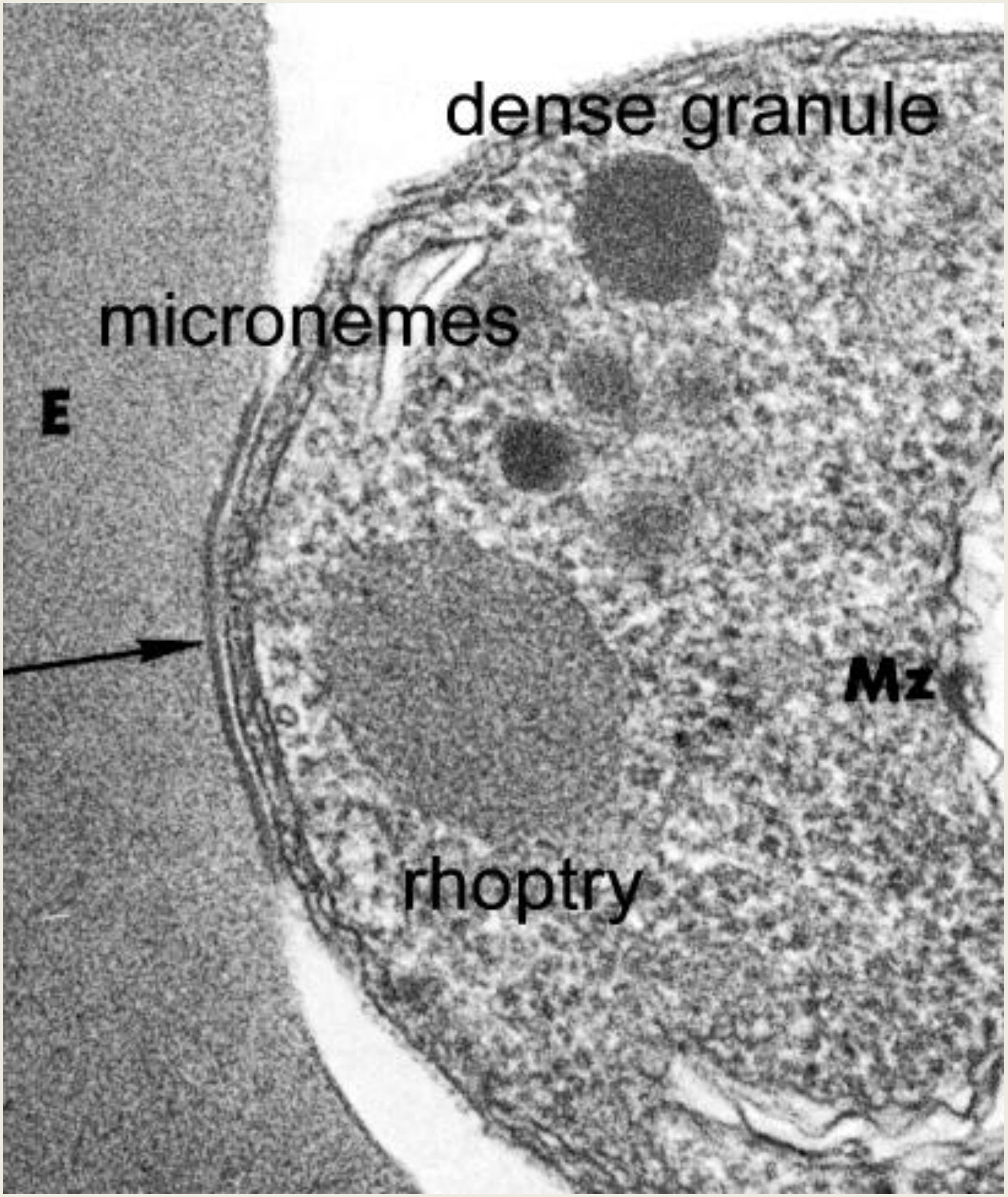
Bannister et al (2003) J Cell Sci 116, 3825

Invasion depends on sequential protein secretion



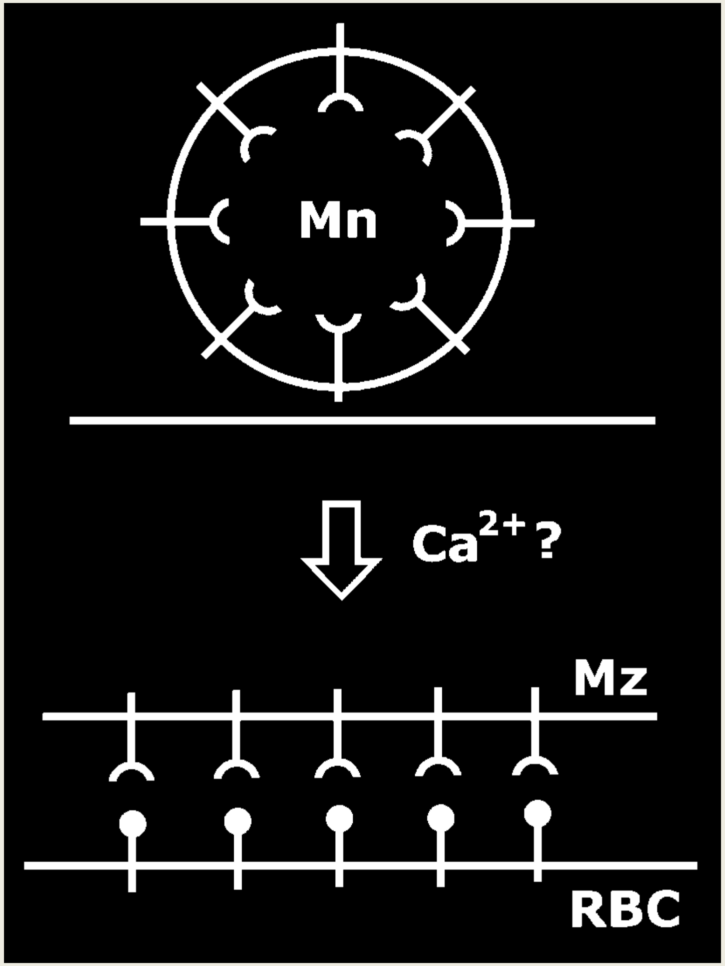
Sultan et al, Cell 90: 511-522

- **Micronemes (Mn): включения с белками (**secretion of micronemes brings protein to the parasite surface that provide ‘traction’ -тяга)
- Microneme proteins are required for **gliding & invasion**, the cleanest example is the *Plasmodium* sporozoite protein TRAP (Mic2 is a *T. gondii* homolog that is functionally equivalent in the tachyzoite)
- Microneme proteins **can bind to a variety of carbohydrates** found on the surface of cells and other biological materials
- Microneme proteins **are assembled into complexes in the ER, mature by proteolysis, are stored in micronemes** and are secreted at the apical tip of the parasite upon stimulation

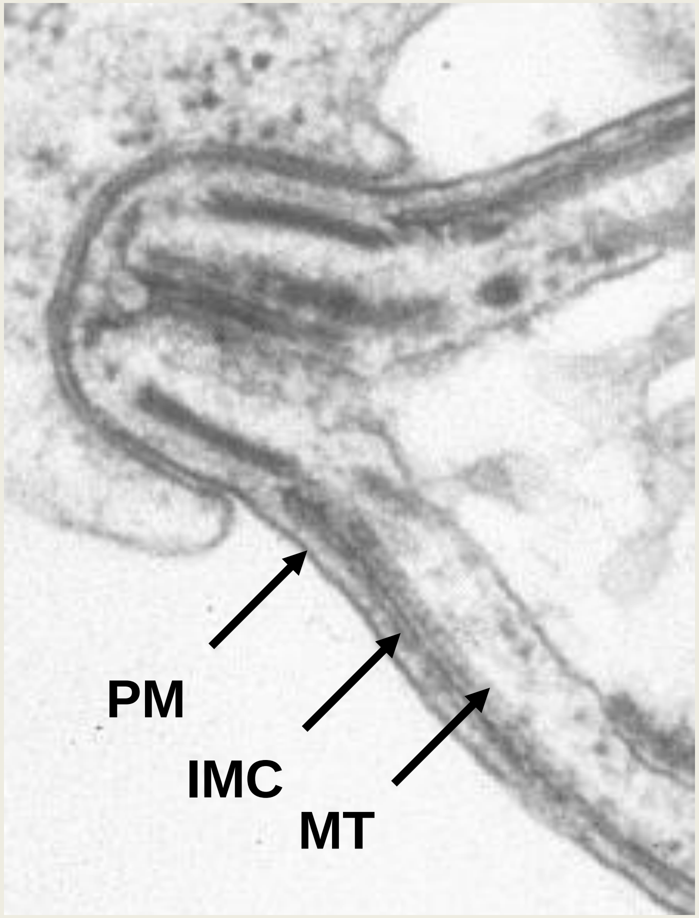


Electron micrograph from Aikawa et al (1978) J. Cell Biol. 77:72

- microneme secretion
- receptor-ligand interactions
- junction formation

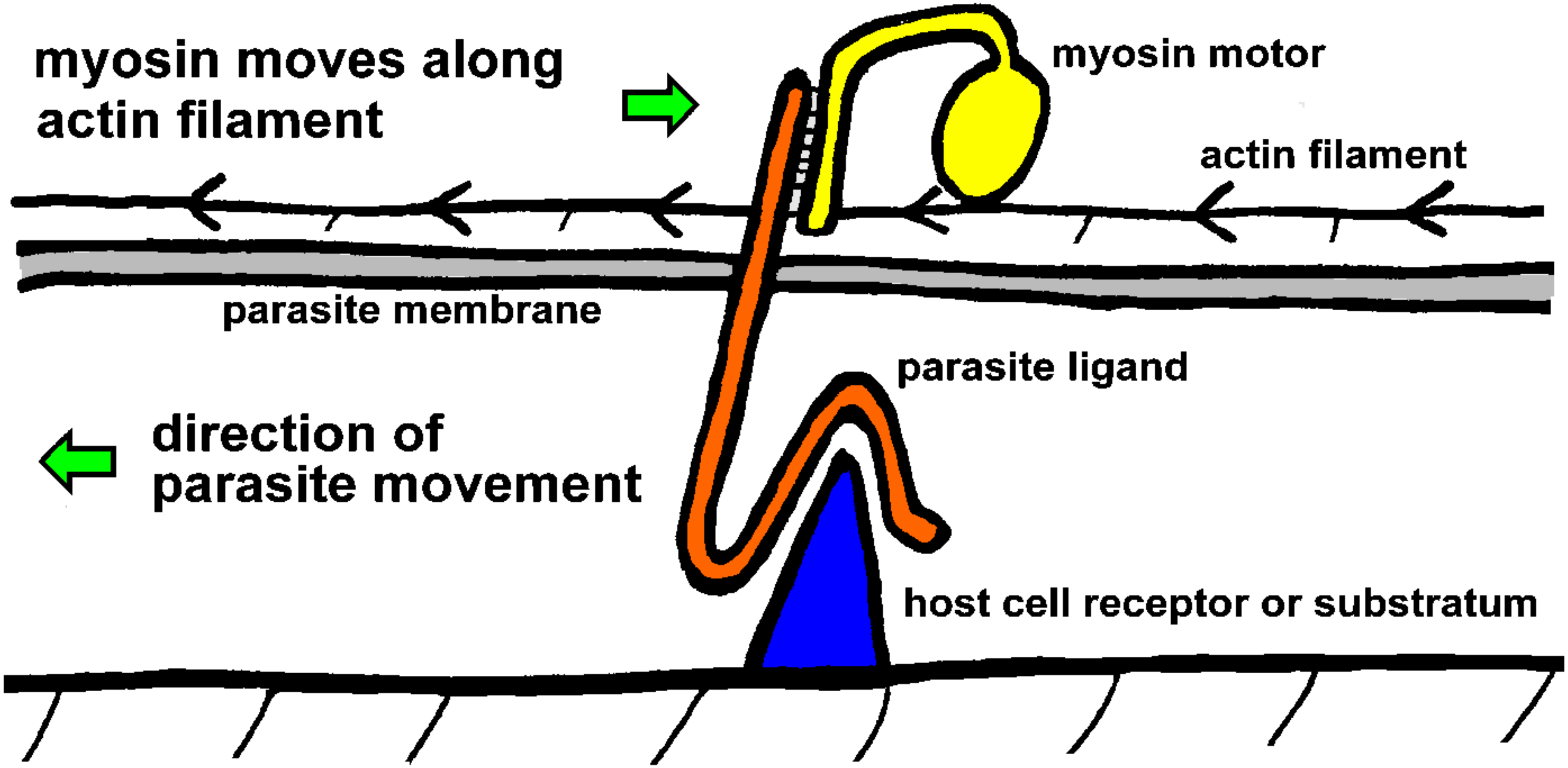


A special myosin is required to move microneme proteins over the surface

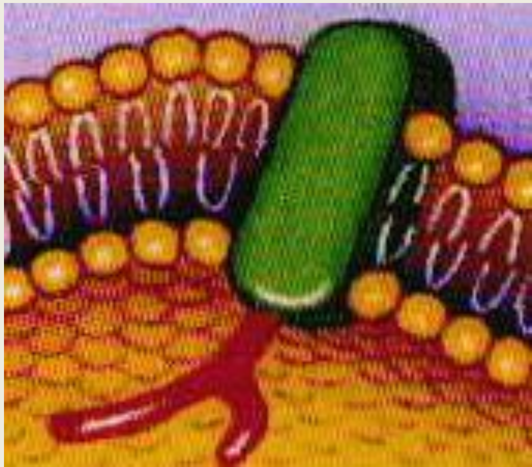


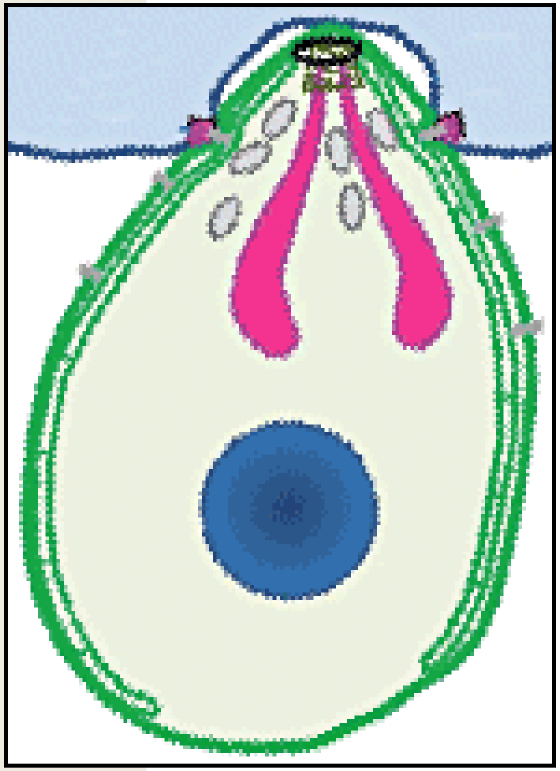
- Toxoplasma and other Apicomplexa **have a parasite specific myosin (MyoA)**
- **This myosin is localized right under the surface membrane of the parasite**
- Using genetic engineering a mutant parasite was constructed in which this myosin can be suppressed
- **Suppression of myosin result in loss of parasite motility (as seen for actin loss of motility also causes loss of host cell invasion)**
- What are the “gears” (*шестеренки*) that connect this motor to the Mic tires and which part is anchored in the parasite?

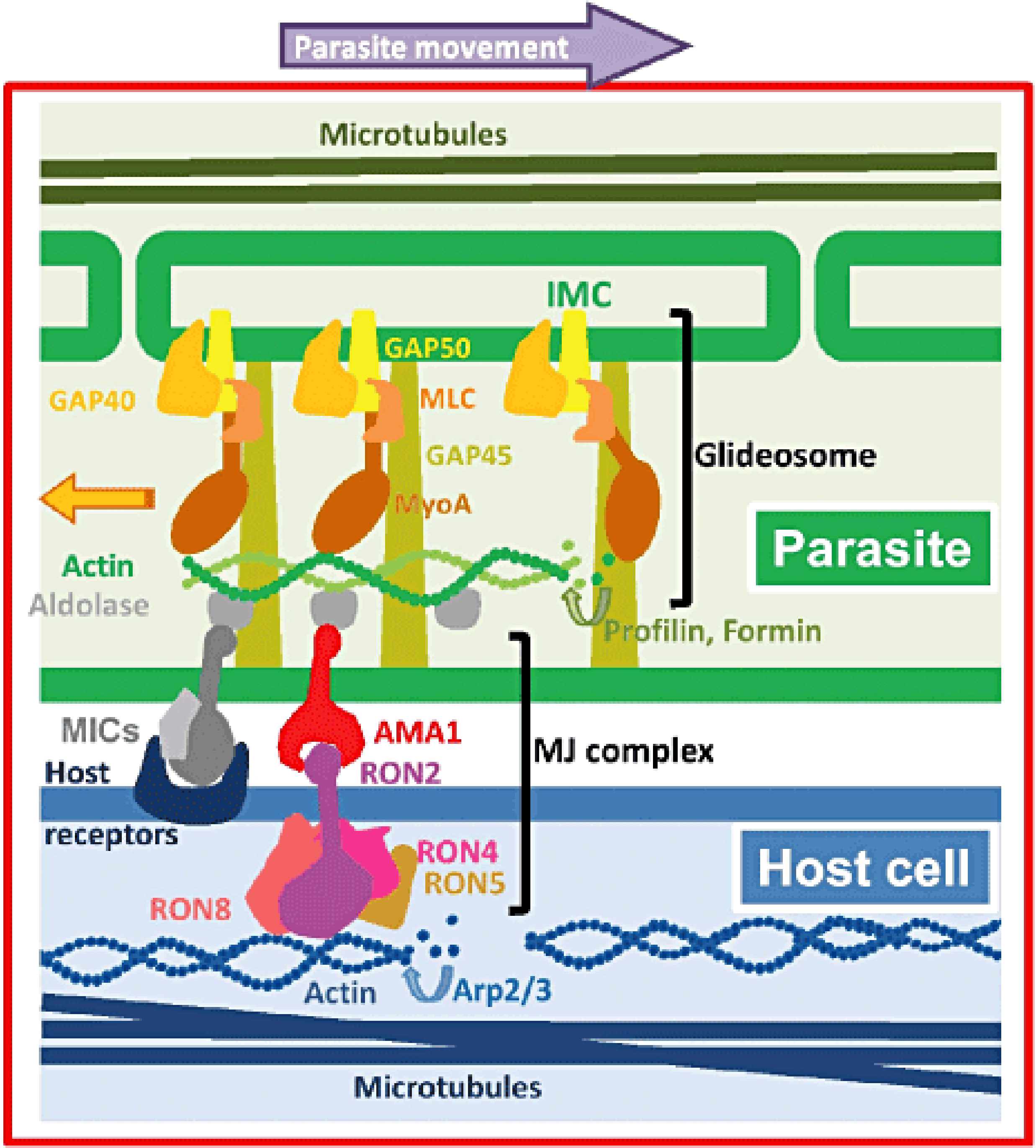
Meissner et al., Science. 298:837-40


myosin moves along actin filament
myosin motor
actin filament
parasite membrane
parasite ligand
direction of parasite movement
host cell receptor or substratum

- **force can be generated by actin/myosin**
- **TRAP necessary for invasion and gliding motility**



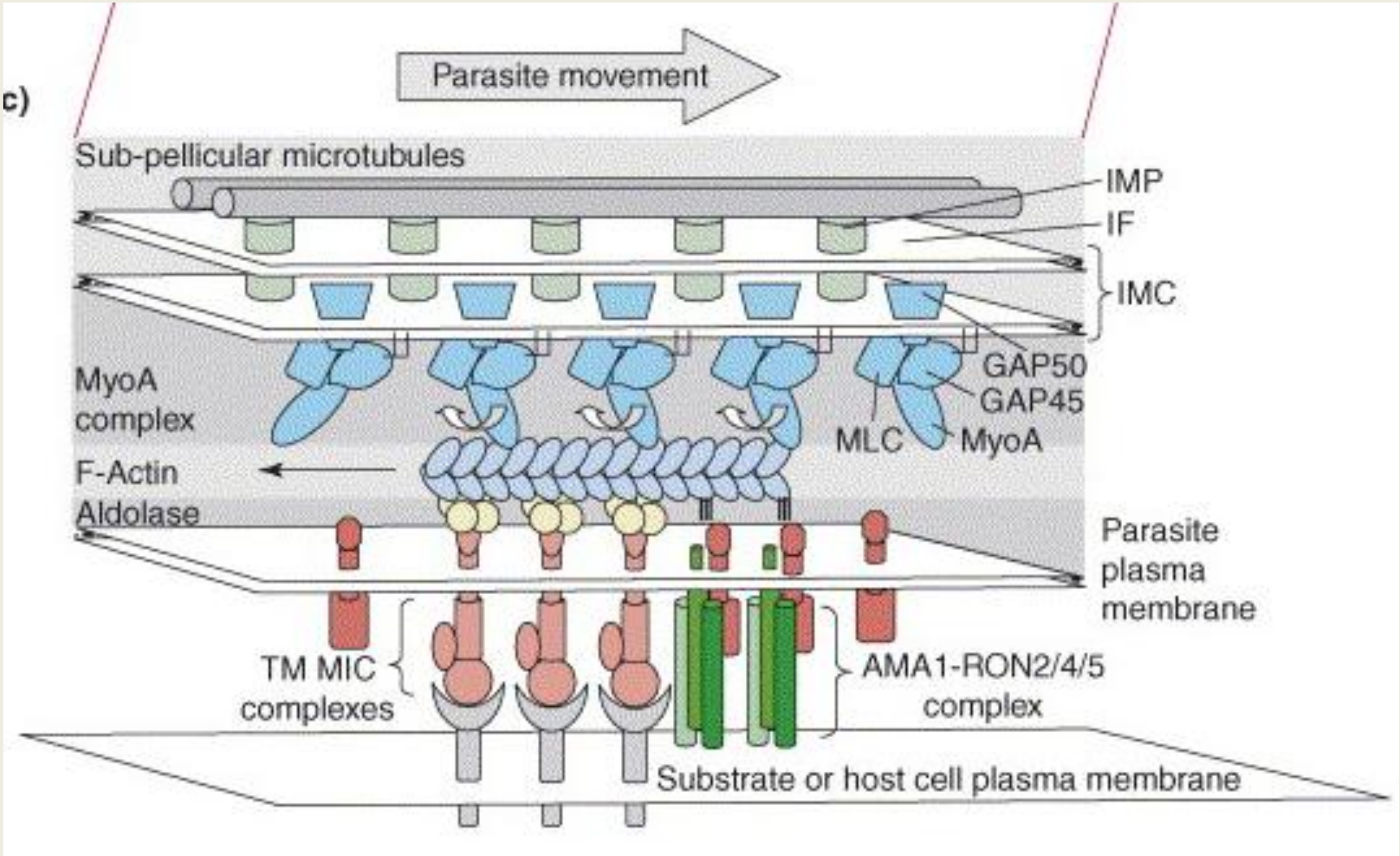




Initial recognition,
attachment & triggering
of rhoptry secretion

Besteiro et al 2011, Cell. Microbiol. 13,797

The apicomplexan engine - the **glideosome** (The treadmilling model of apicomplexan gliding motility)



The conveyor-belt model of gliding motility

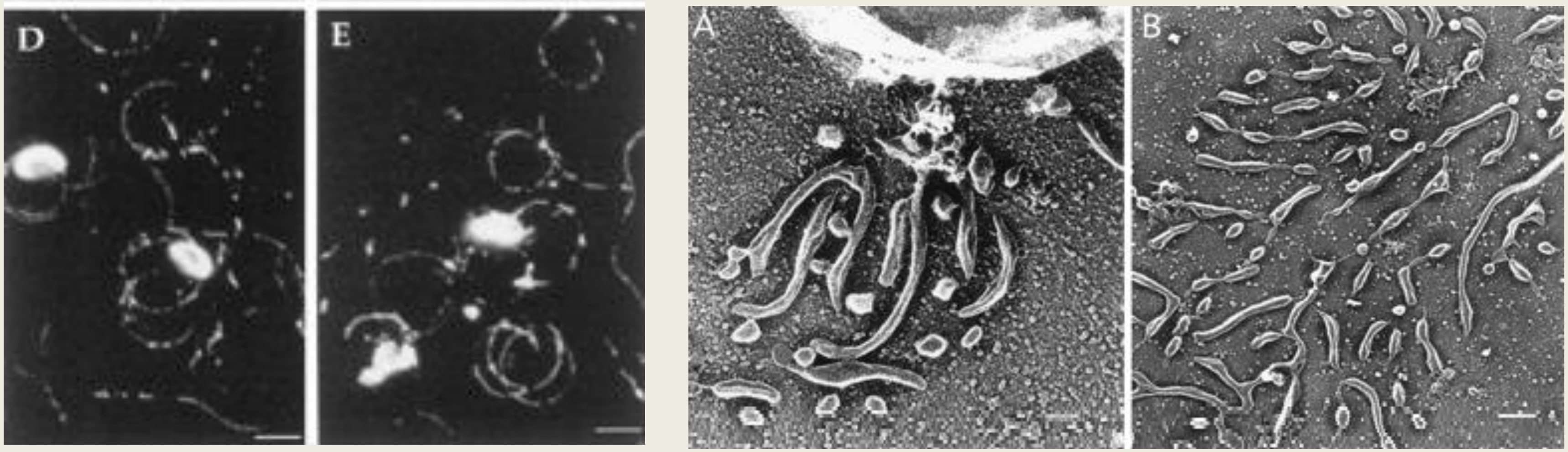
Movies by Dr. C. King (University College, London)

- **Gregarines are a group of ‘primitive’ apicomplexans which parasitize invertebrates**
- In comparison ot Toxoplasma or the malaria parasite these are fairly large cells which makes them easier to study

The conveyor-belt model of gliding motility

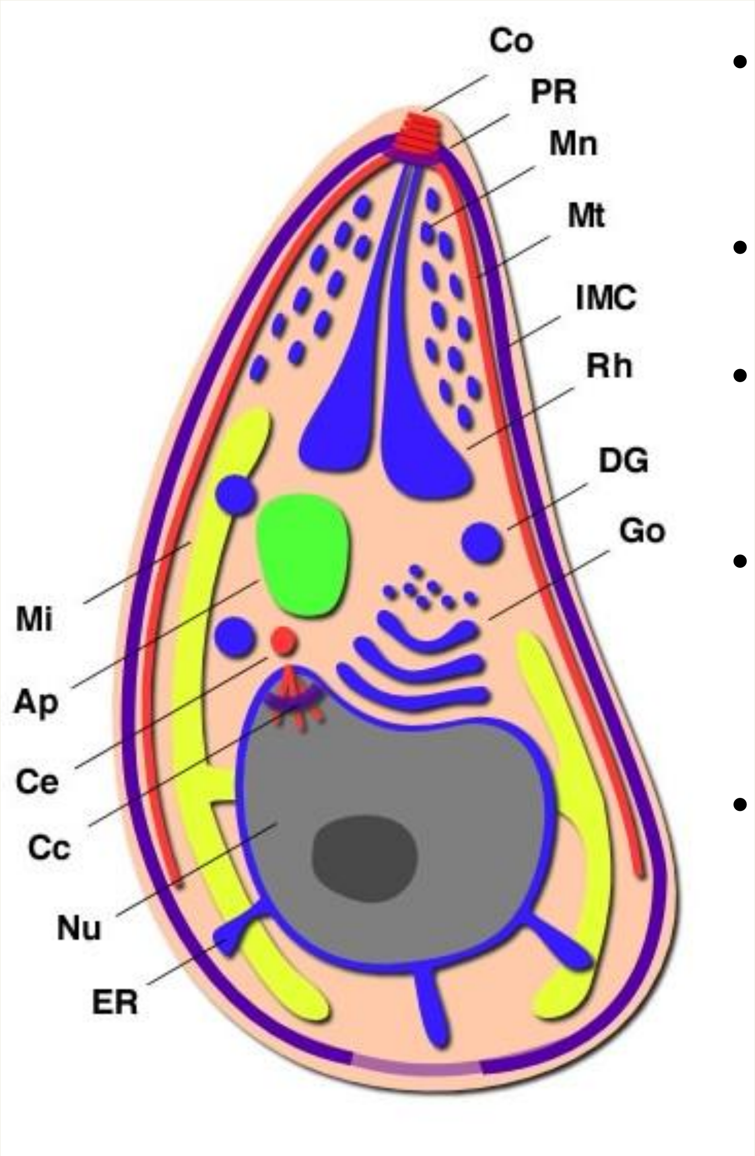
- **Beads (капли)** attached to the surface of gregarines are ‘treadmilled’ to the end of the parasite cells
- This suggests that the **gliding machine moves microneme proteins over the surface from the apical to the basal end of the parasite**

Gliding parasites deposit proteins and lipid trails



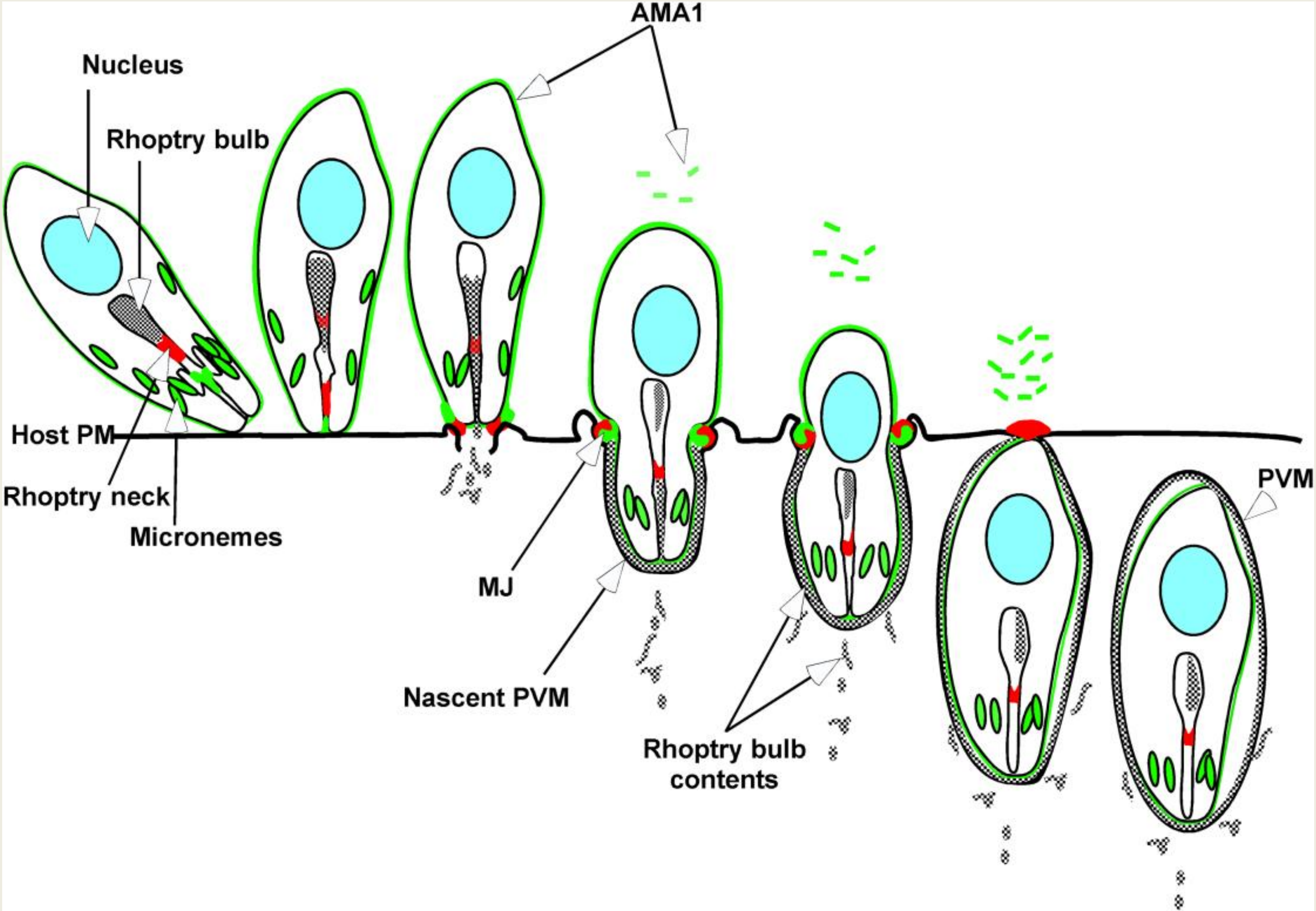
- Similar to a slug the parasite leaves behind a trail of surface proteins and lipids
- **A protease (sheddase) cleaves microneme proteins at the end of the cell to detach and allow for propulsion (толчок)**
- What is the force driving microneme proteins -- actin polymerisation or an actin/myosin motor?

Secretion of the rhoptries is associated with PV formation (PV =parasitophorous vacuole)

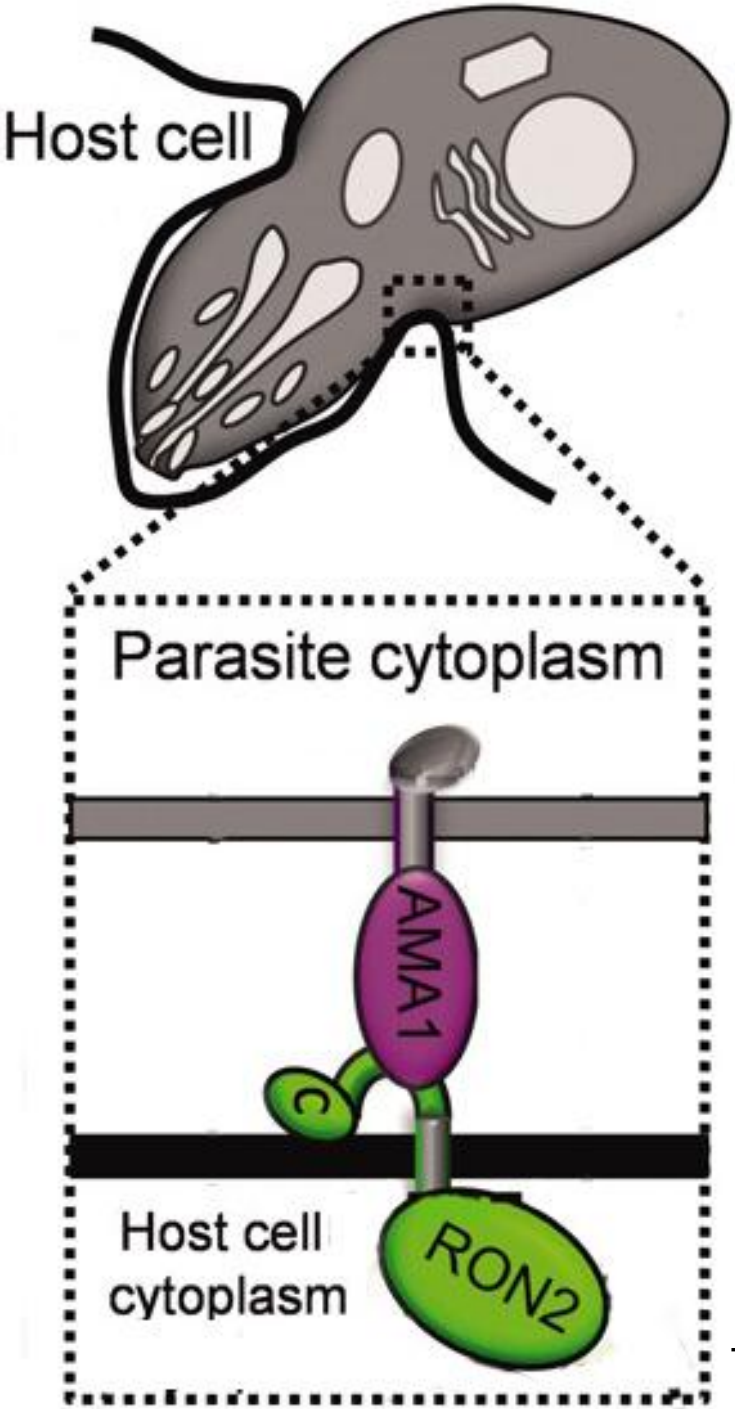


- **Rhoptries (Rh):** secretion of rhoptries is required for the formation of the parasitophorous vacuole
- Like micronemes rhoptries are secreted at the apical tip of the parasite
- Some rhoptry proteins **make up (part) of the moving junction** (they are stored in the neck portion of the organelles and called RONs)
- Other rhoptry proteins found throughout the **membrane of the parasitophorous vacuole** after secretion and are stored in the bulbous part (ROPs)
- A third group of very interesting rhoptry proteins **is injected into the host cell and manipulates gene expression in the host nucleus**

Rhoptry proteins have multiple functions in vacuole formation

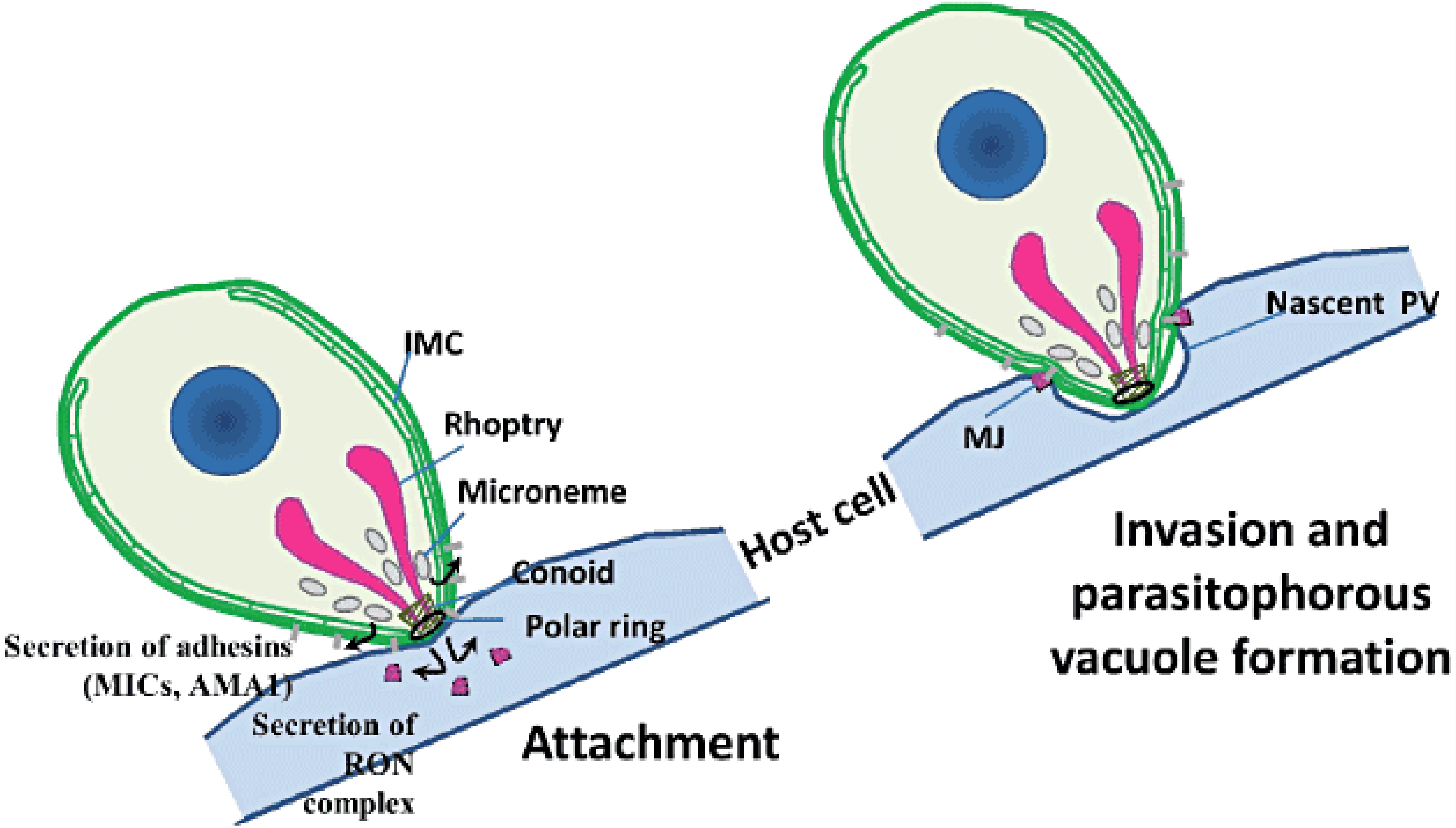


Rhoptries also participate in junction formation



- Rhoptry neck proteins (RONs) inserted into host membrane
- RON2 interacts with AMA-1
- Forms part of the moving junction

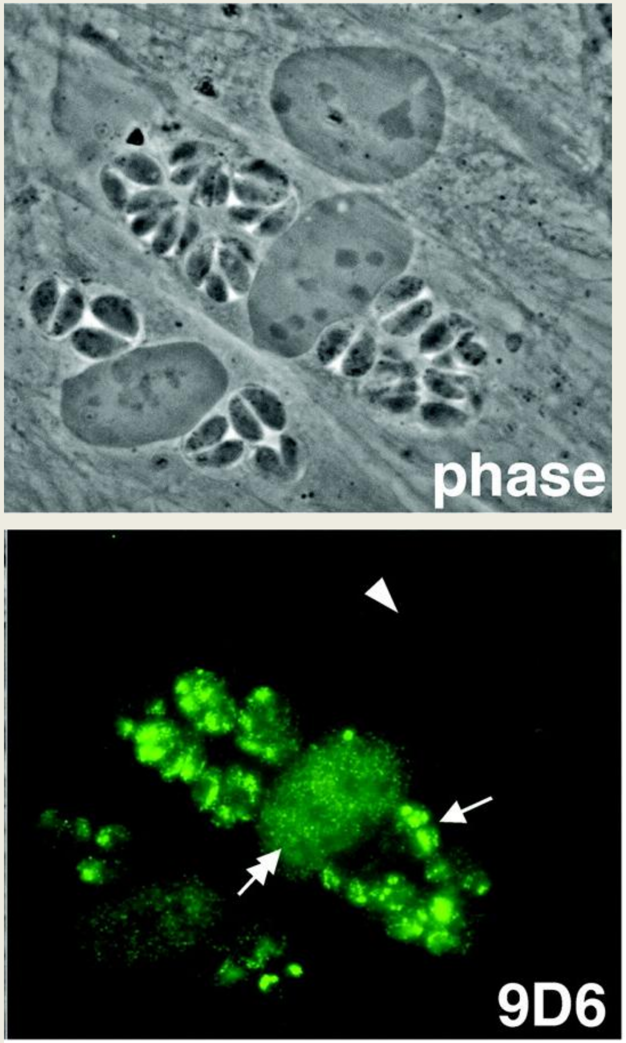
Tonkin et al 2011, Science 233,463


IMC
Rhoptry
Microneme
Conoid
Polar ring
Secretion of adhesins (MICs, AMA1)
Secretion of RON complex
Attachment
Host cell
Nascent PV
MJ
Invasion and parasitophorous vacuole formation

Where does the membrane for the parasitophorous vacuole come from?

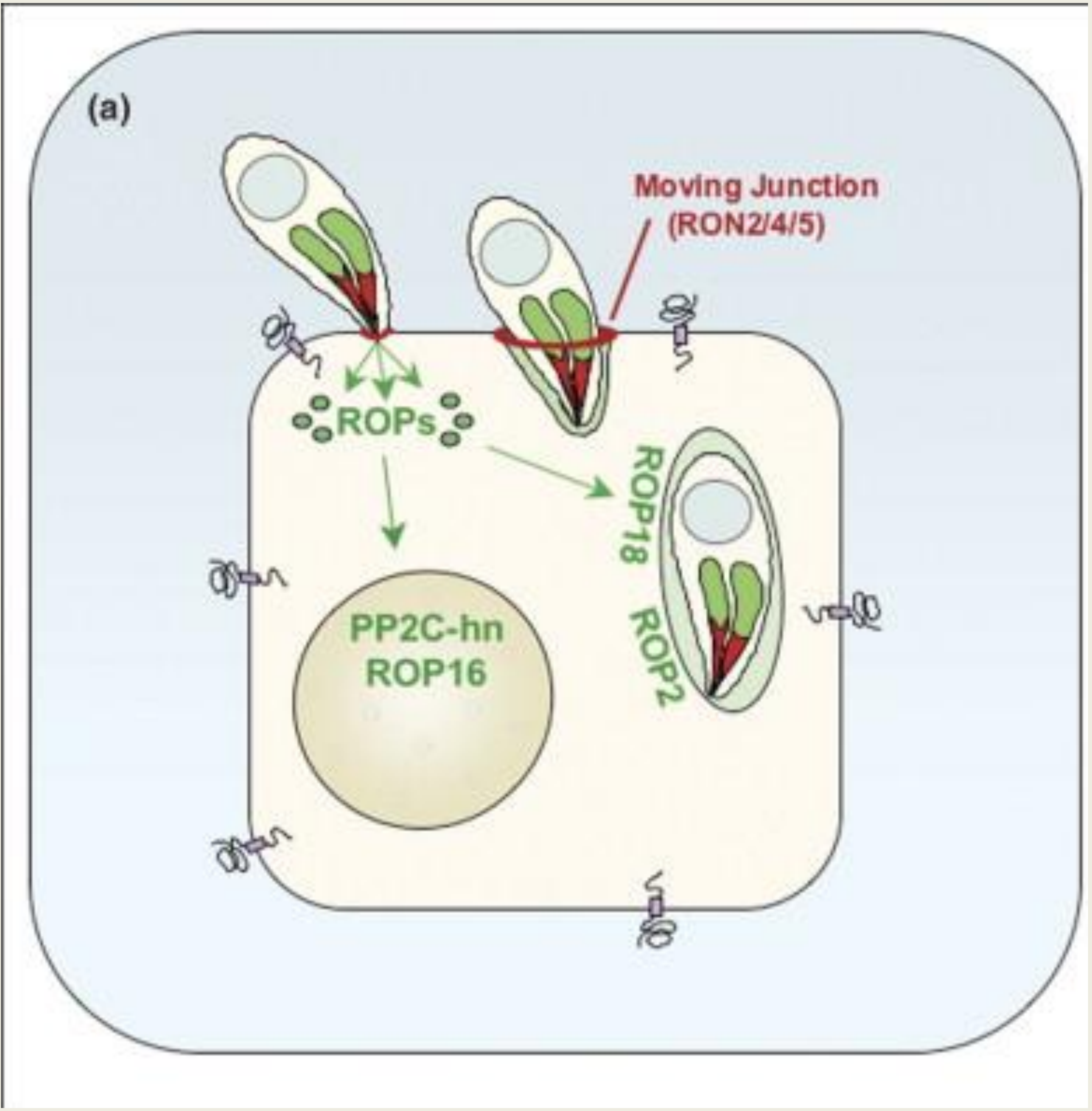
- The PV membrane is derived from the host cell plasma membrane
- The PV is provided by the parasite (e.g. by secretion from the rhoptries)
- **Both contribute to the PV**

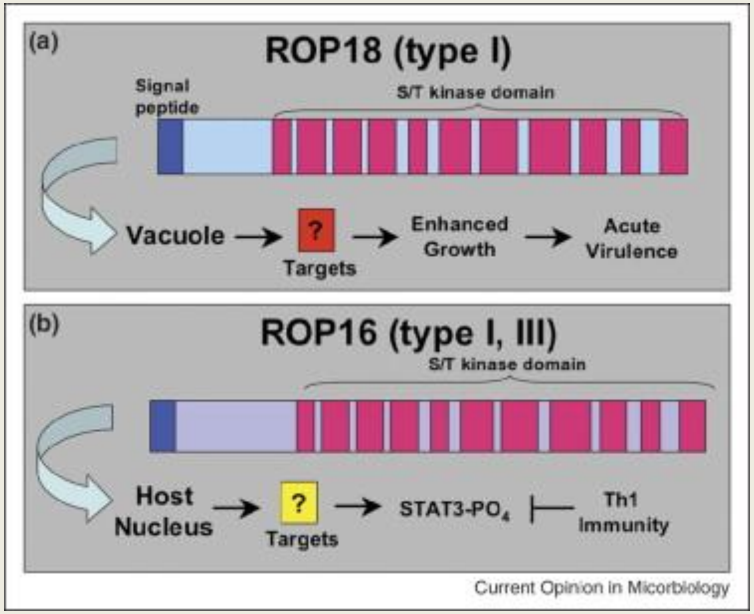
Rhoptry proteins modify the function of the host cell nucleus



- Several rhoptry proteins are injected into the host cell cytoplasm during invasion
- They accumulate in the host cell nucleus
- Interestingly, many of them are enzymes capable of changing the phosphorylation state of proteins (kinases & phosphatases)
- Their precise function remains to be determined but it appears that they modulate gene expression in the host cell and that their activity is required for rapid growth and the ability to cause disease (virulence)

Rhoptry proteins have multiple functions in vacuole formation & host manipulation

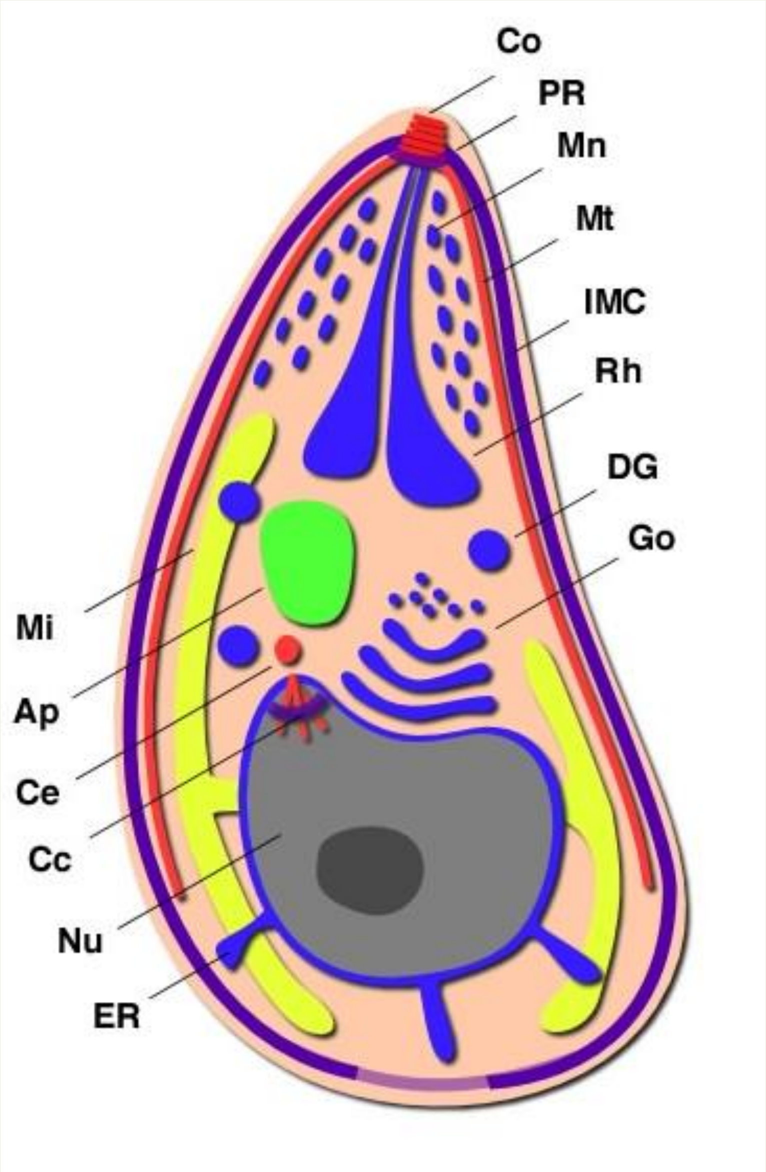




Current Opinion in Micorbiology

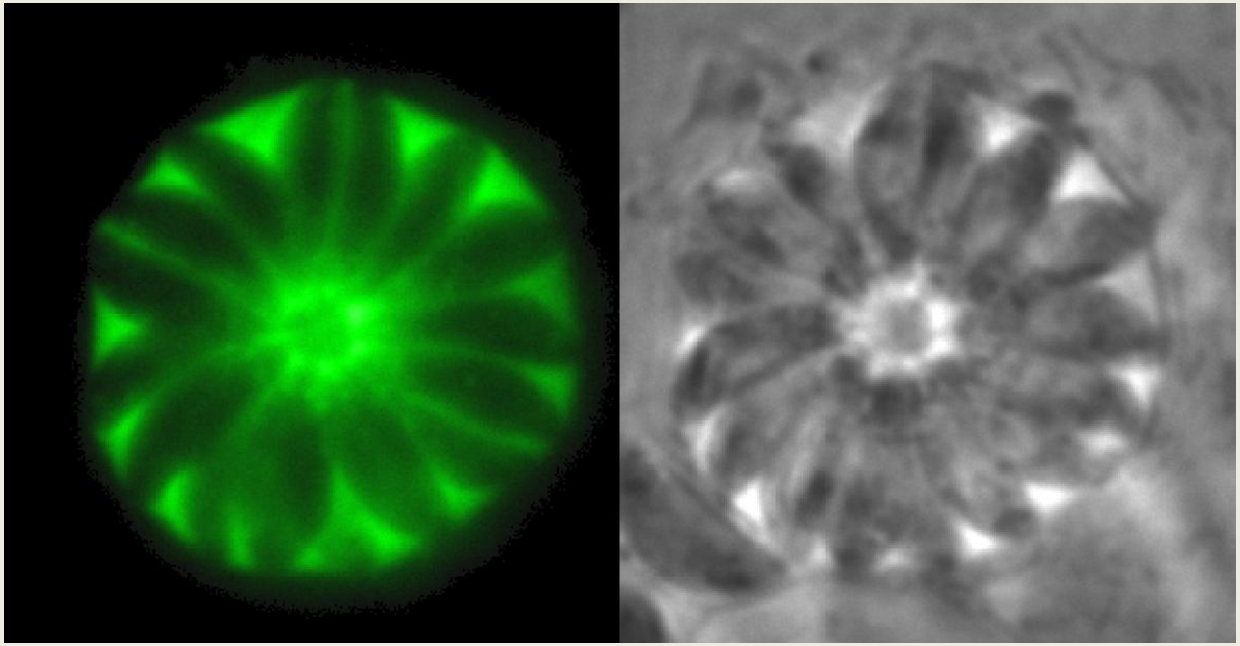
Bradley & Sibley, Current Opinion in Microbiology 10: 582-587

Invasion depends on sequential protein secretion



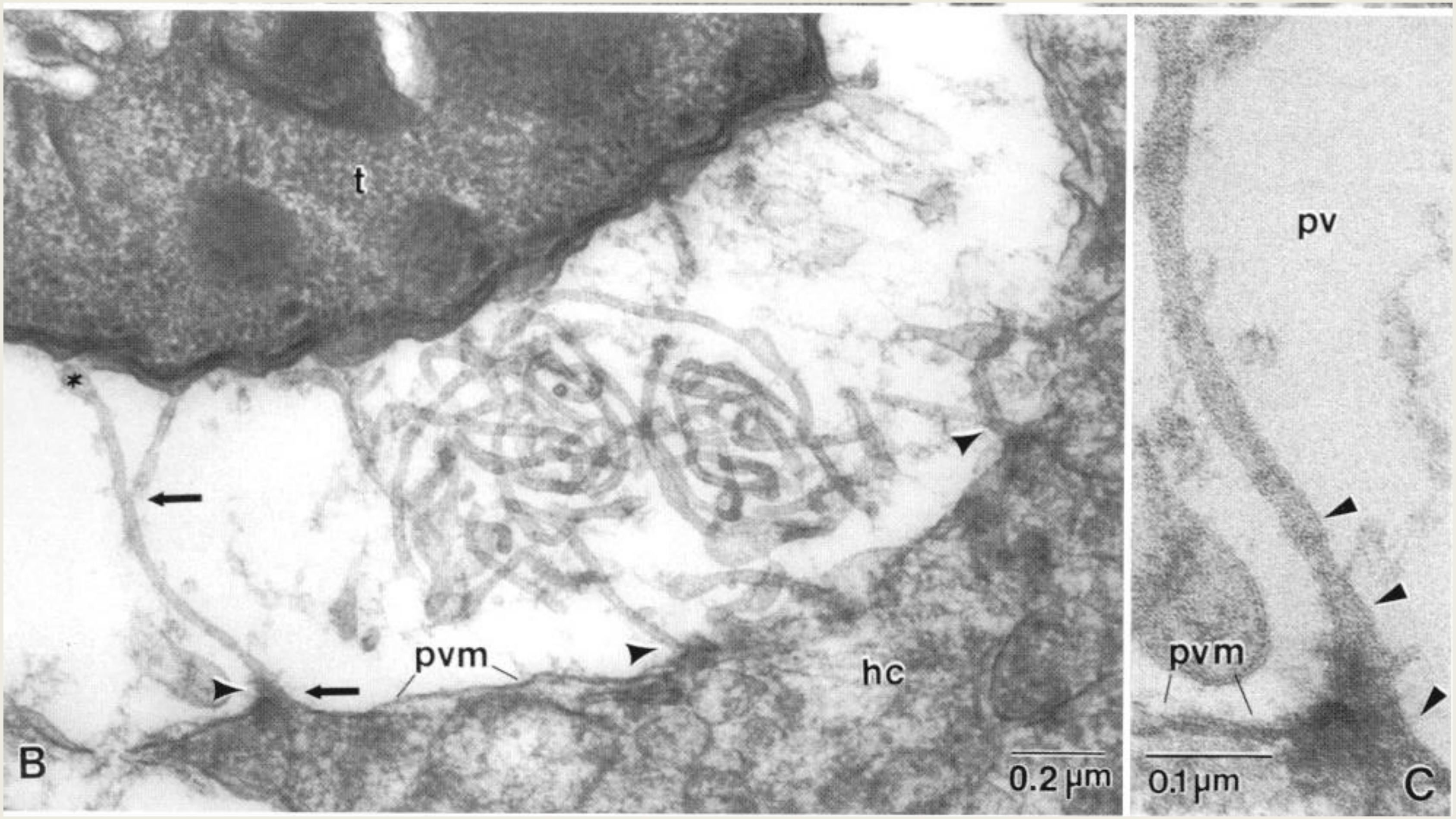
- **Dense granules (DG):** secretion of dense granules occurs after the vacuole is fully formed and continues throughout the intracellular growth of the parasites
- Dense granules are secreted from the basal end of the parasites
- Dense granule proteins likely play a **role in modification of the vacuole into an environment supportive of parasite growth**

Dense granule proteins are secreted into the PV (parasitophorous vacuole)



- **Certain dense granule proteins are soluble in the lumen of the PV others integrate into the membrane**
- **These proteins are probably involved in modifying the vacuole**

Dense granules are involved in establishing the intravacuolar network

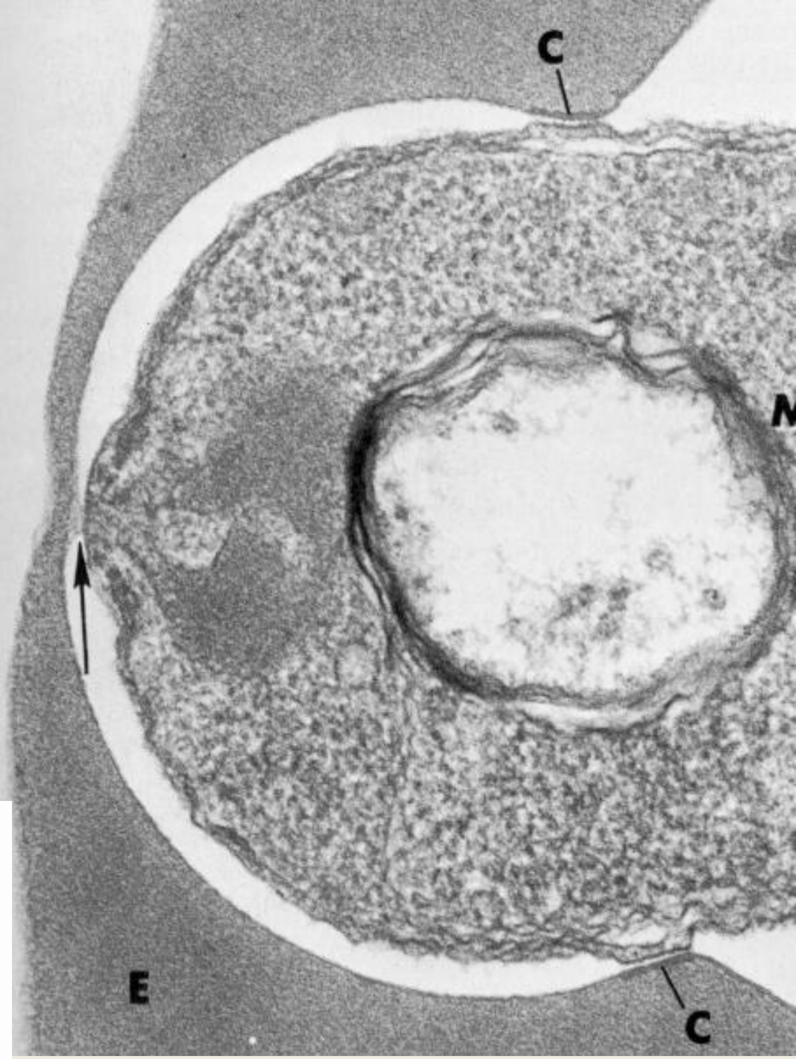


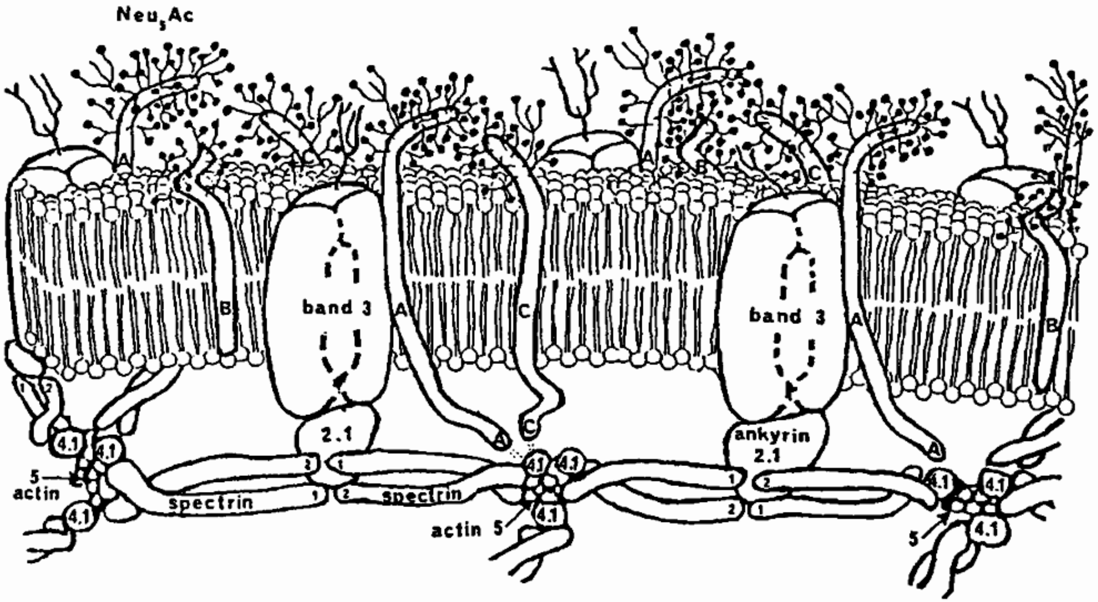
Plasmodium Merozoite invasion: a complex and ordered process

- Initial Binding
  - merozoite surface proteins (eg. MSP-1)
- Reorientation (AMA-1)
- Microneme Discharge and Junction Formation
  - Ca^{2+} signal?
  - receptor-ligand interactions (adhesive proteins)
- Rhoptry Discharge and Vacuole Formation
  - clearing of host membrane proteins
  - moving junction formation (RON2/AMA-1)
- Parasite Entry
  - mediated by actin-myosin ‘glidosome’
- Closure of PVM and Erythrocyte Membrane

Events correlated with entry

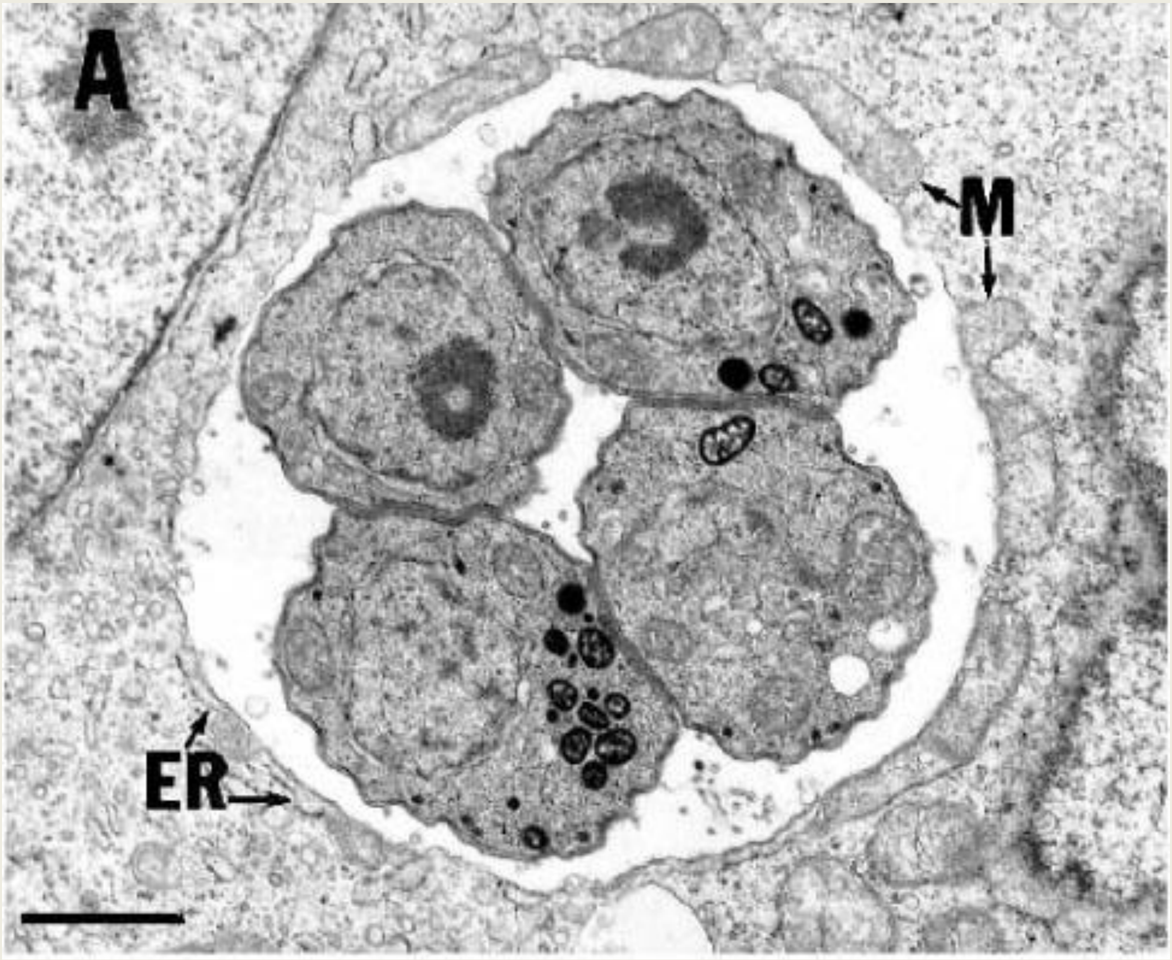
- clearance of erythrocyte membrane proteins
- host membrane invagination
- parasitophorous vacuolar membrane (PVM) formation



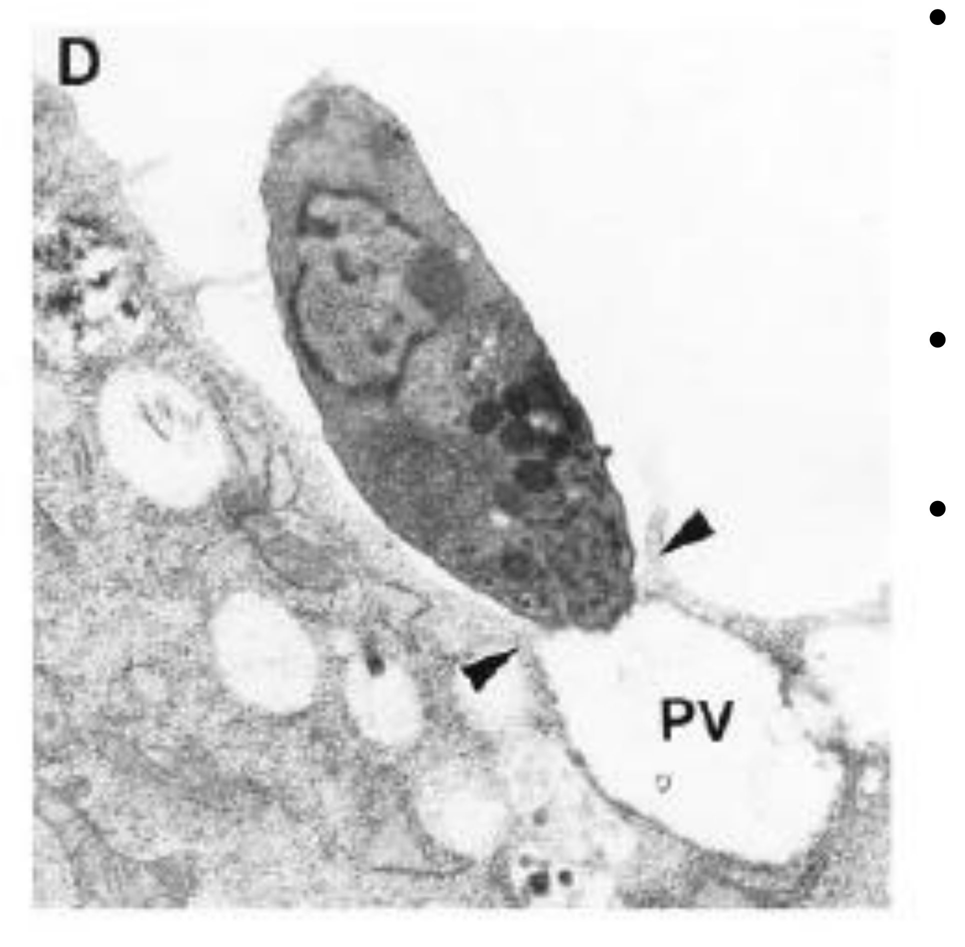


- junction becomes an annulus (ring)

Host cell mitochondria and ER are recruited to the PVM



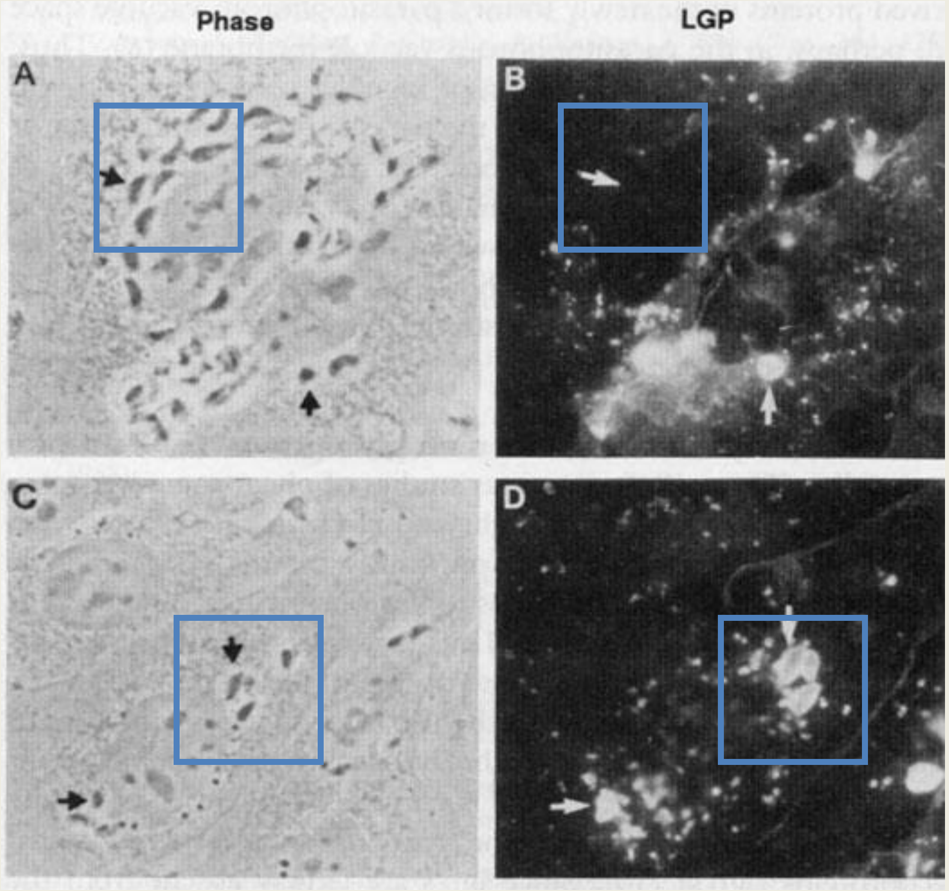
Invasion depends on parasite not host cell actin



- **Cytochalasin** treatment (inhibitor of actin polymerization) does not to appear to inhibit attachment
- CytD **inhibits the movement of the parasite into the host cell.**
- **A parasitophorous vacuole (PV)** is still set up, however the **parasite can not move in**, and the moving junction remains at the apical tip of the parasite

Dobrowolski JM, Sibley LD. Cell. 1996 84(6):933-9.

The parasitophorous vacuole is not fusing with lysosomes



- **Macrophages were incubated with life (A/B) and heat killed (C/D) parasites**
- **only vacuoles containing heat killed parasites show staining for a lysosomal marker protein.**
- **Dead parasites go in by phagocytosis, living parasites enter differently**

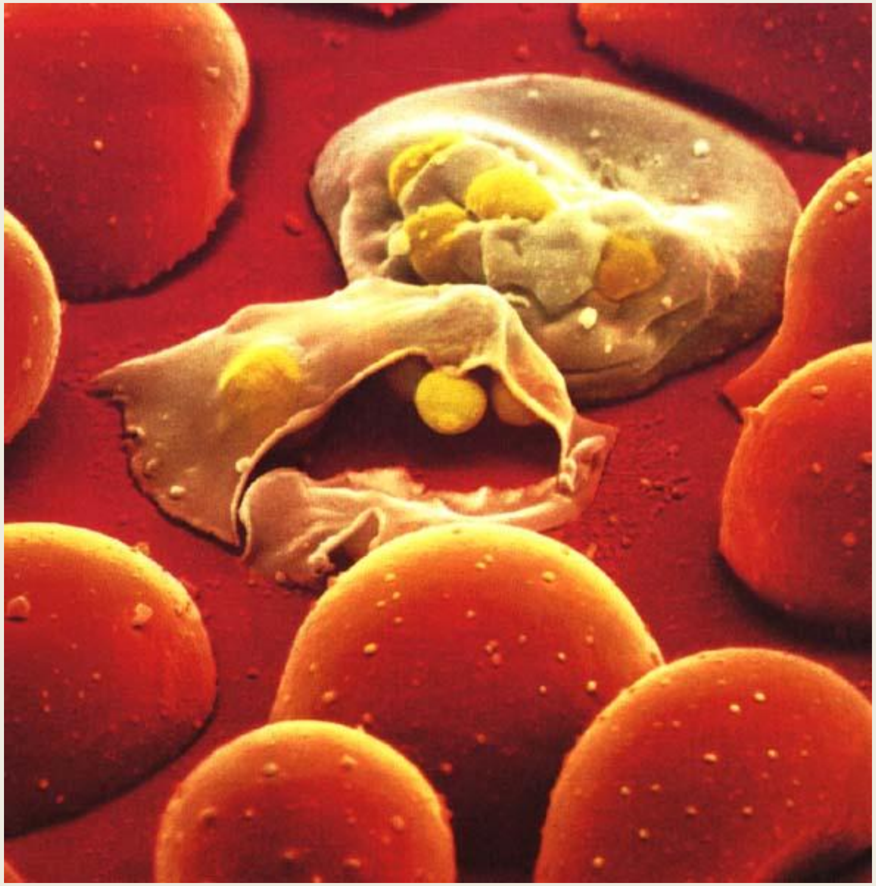
Joiner et al., Science 249:641-6

Falstatin

- Falstatin is a **cysteine protease inhibitor** of *Plasmodium falciparum.*
- The functions of proteases in the erythrocyte invasion pathway include the "breakdown of red cell hemoglobin, the release of parasites from red cells, and the invasion of red cells by free parasites" (Pandey et al.).
- Falstatin is found to be a potent reversible inhibitor of the P. falciparum cysteine proteases. Thus, P. falciparum **requires expression of falstatin to limit proteolysis by certain host or parasite cysteine proteases during erythrocyte invasion**
- **Red cell invasion is inhibited when falstatin is inhibited**; this is true because cysteine proteases are able to work to restrict erythrocyte invasion when falstatin is inhibited.

Apicomplexan invasion

- Active, parasite driven process
- Depends on parasite actin/myosin motility (conveyor belt model=модель ленточного конвейера)
- Involves secretion of micronemes (attachment, motility), rhoptries (parasitophorous vacuole & moving junction formation) and dense granules (makes PV into a suitable home)
- Sets up a parasitophorous vacuole which initially is derived from the host cell-membrane
- A moving junction is formed which screens out host membrane proteins from the PV, the PV is fusion incompetent and the parasite protected

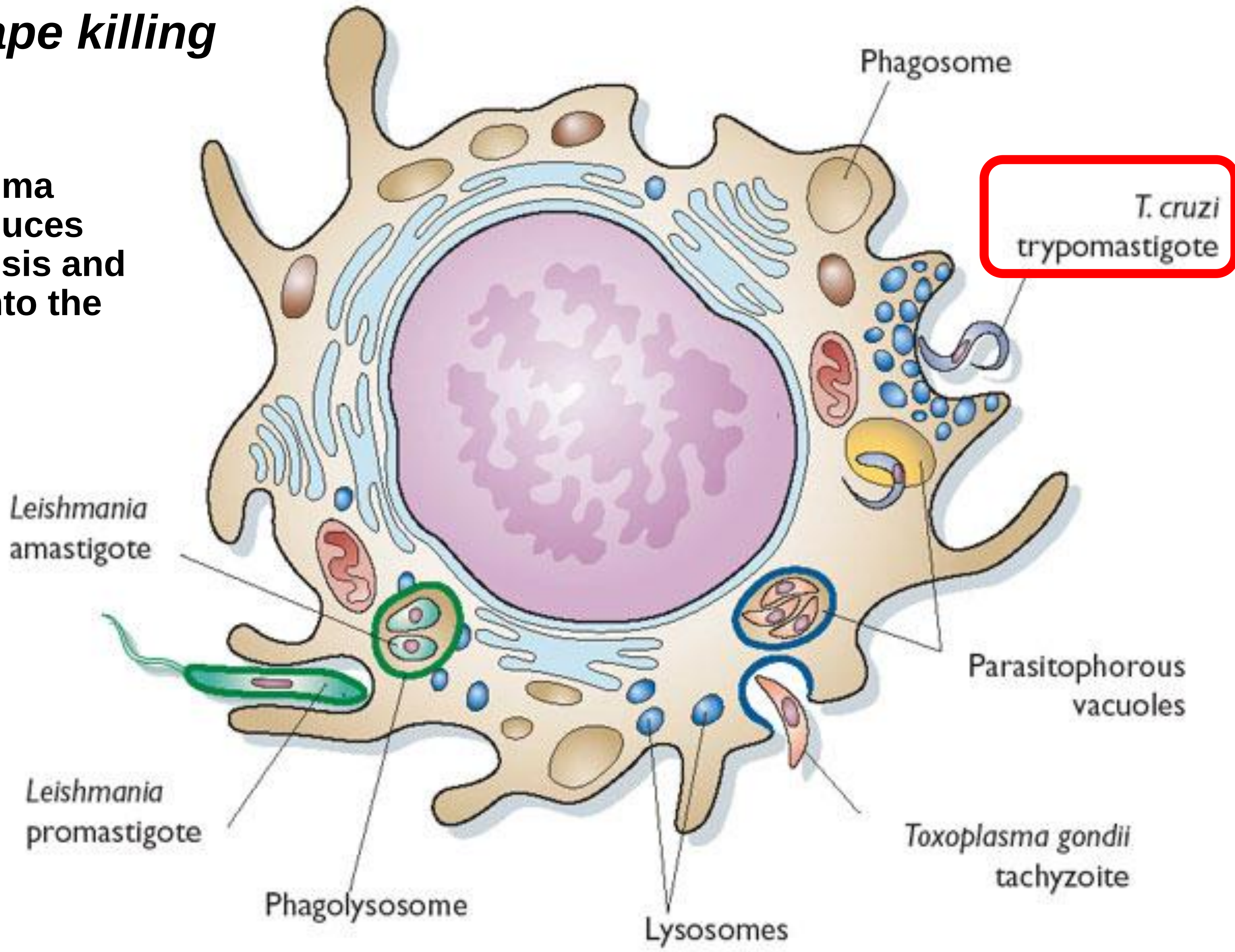


- **Malaria** is caused by the invasion of human erythrocytes (red blood cells) by a protozoan parasite.
- The most virulent and severe form of malaria is caused by ***Plasmodium falciparum***.
- The merozoites of the protozoan are the actual invaders of the erythrocytes.
- Merozoites are the daughter cells of the protozoan parasite.

Strategies of Intracellular parasitism

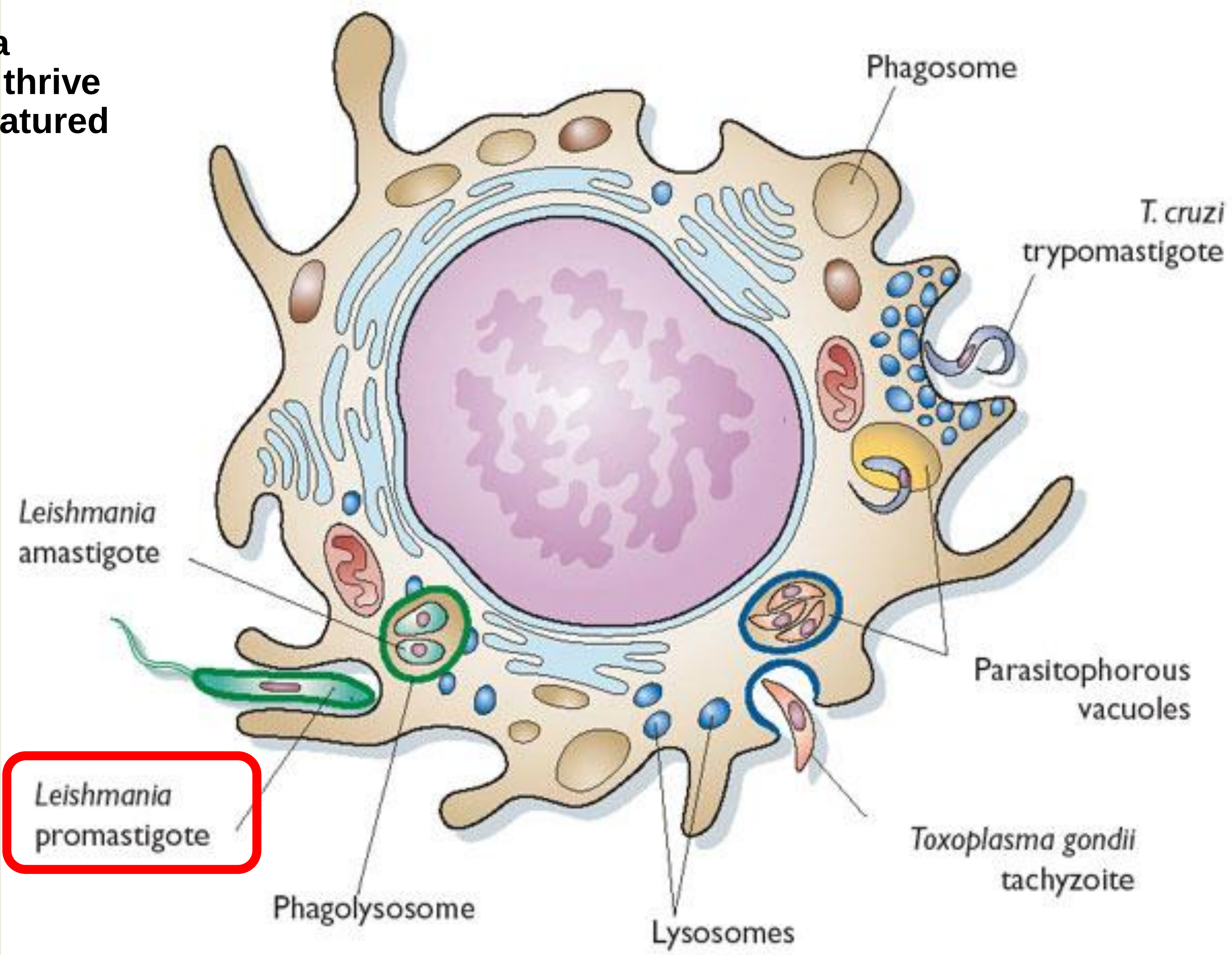
to escape killing

Trypanosoma cruzi -- induces phagocytosis and escapes into the cytoplasm


Phagosome
T. cruzi trypomastigote
Leishmania amastigote
Parasitophorous vacuoles
Leishmania promastigote
Toxoplasma gondii tachyzoite
Phagolysosome
Lysosomes

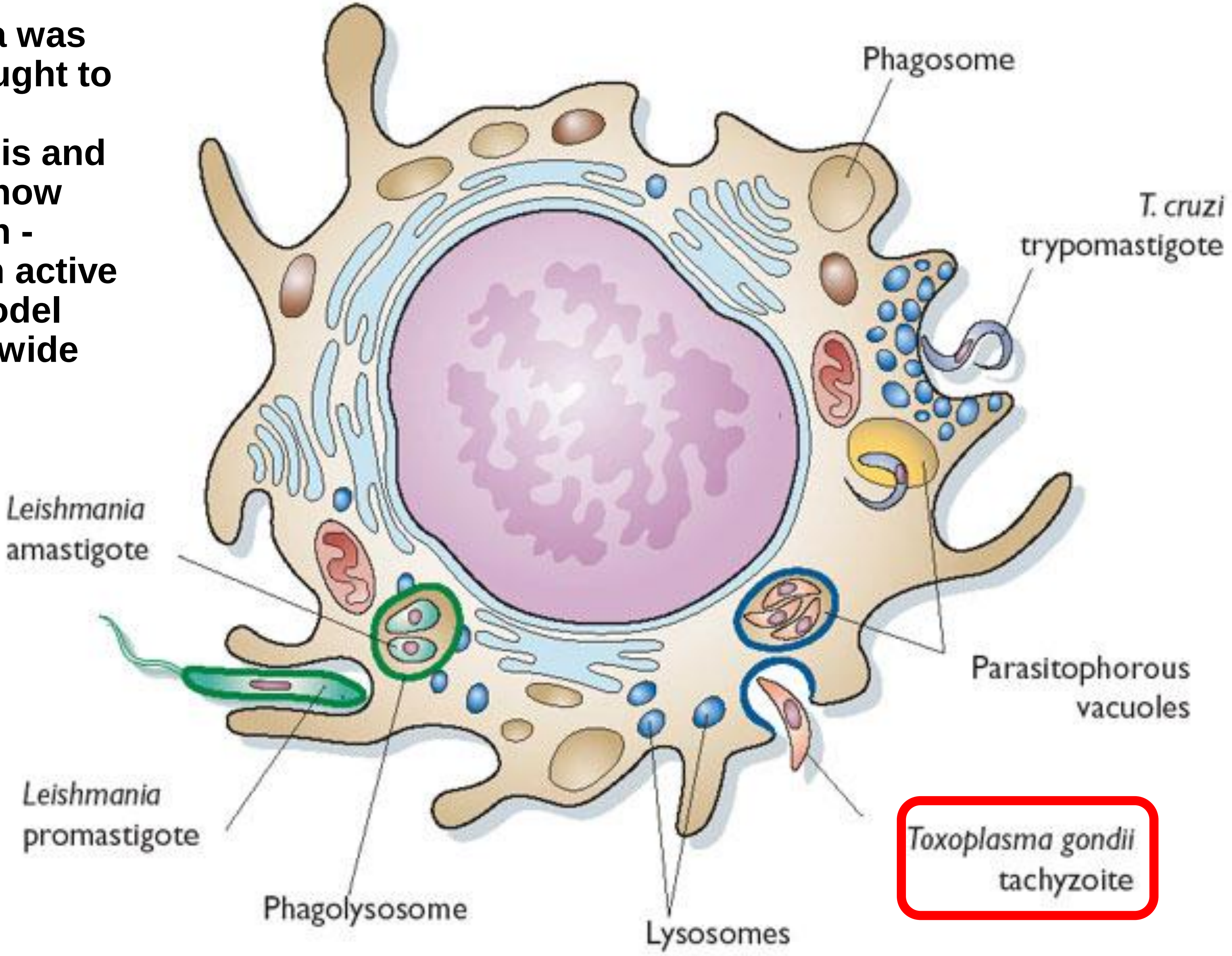
Strategies of Intracellular parasitism

Leishmania appears to thrive in a fully matured lysosome



Strategies of Intracellular parasitism

Toxoplasma was equally thought to induce phagocytosis and then some how block fusion - however, an active invasion model has gained wide acceptance



Phagosome
T. cruzi trypomastigote
Leishmania amastigote
Parasitophorous vacuoles
Leishmania promastigote
Toxoplasma gondii tachyzoite
Phagolysosome
Lysosomes